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(54) **Plants with modified flowers**

Pflanzen mit modifizierten Blüten

Plantes à fleurs modifiées

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Description

[0001] This invention relates to a method of restoring fertility to a transgenic nuclear male-sterile or female-sterile plant by crossing such a sterile plant with a transgenic fertility-restorer plant to provide a transgenic fertility-restored plant having a foreign DNA sequence from the nuclear genome of the restorer plant that is stably integrated into the nuclear genome of the restored plant. The foreign DNA sequence of this invention contains a foreign DNA (hereinafter the "fertility-restorer DNA") that: 1) encodes a first protein or polypeptide which is an inhibitor of a ribonuclease and which, when produced or overproduced in a cell of a flower, particularly a male or female reproductive organ thereof, or a seed or an embryo of the restored plant, prevents the activity in the flower, seed or embryo cell of a second protein or polypeptide which is the ribonuclease and which, when produced or overproduced in the flower, seed or embryo cell, would otherwise significantly disturb adversely the metabolism, functioning and/or development of the flower, seed or embryo cell; and 2) is in the same transcriptional unit as, and under the control of, a first promoter which is capable of directing expression of the fertility-restorer DNA at least in the same flower or seed or embryo cells of the restored plant where the second RNA, protein or polypeptide is being produced or overproduced. The second, protein or polypeptide is encoded by a foreign "sterility-DNA" that is from the nuclear genome of the sterile plant, that is also stably integrated into the nuclear genome of the restored plant and that is under the control of a "sterility-promoter" which is capable of: i) directing expression of the sterility DNA selectively in specific cells of each flower, particularly at least one male or at least one female reproductive organ thereof, or each seed or each embryo of the restored plant and ii) thereby rendering the restored plant male- or female-sterile in the absence of expression of the fertility-restorer DNA in the specific flower, seed or embryo cells.

[0002] The foreign DNA sequence of this invention, transferred from the restorer plant into the restored plant, is optionally a foreign chimaeric DNA sequence that can also contain a second foreign DNA (the "first marker DNA") that: 1) encodes a third RNA, protein or polypeptide which, when present at least in a specific tissue or specific cells of the plant, renders the entire plant easily separable or distinguishable from other plants that do not contain the third RNA, protein or polypeptide at least in the specific tissue or specific cells of the plant; 2) is in the same transcriptional unit as, and under the control of, a second promoter which is capable of directing expression of the first marker DNA in at least specific tissue or specific cells of the plant; and 3) is in the same genetic locus of the nuclear genome of the restored plant as the fertility-restorer DNA.

[0003] This invention also relates to a foreign chimaeric DNA sequence that contains at least one fertility-restorer DNA under the control of at least one first promoter and that can also contain, adjacent to the fertility-restorer DNA(s) and the first promoter(s), at least one first marker DNA under the control of at least one second promoter.

[0004] This invention further relates to: a vector that contains the foreign DNA sequence of this invention and is suitable for the transformation of a plant cell, whereby the foreign DNA sequence is stably integrated into the nuclear genome of the cell; the resulting fertility-restorer plant cell; cultures of such fertility-restorer plant cells; a fertility-restorer plant and its reproductive material (e.g., seeds) which can be regenerated from such a fertility-restorer plant cell and the nuclear genome of which contains, stably integrated therein, the foreign DNA sequence; a fertility-restored plant and its reproductive material containing, stably integrated in their nuclear genome, the foreign DNA sequence, together with at least one sterility DNA under the control of at least one sterility promoter; and a cell of the fertility-restored plant, as well as cultures thereof.

[0005] This invention yet further relates to a process for producing the restorer plant and its reproductive material by transforming a cell of the plant with the foreign DNA sequence whereby the fertility-restorer DNA is: 1) under the control of the first promoter and optionally in the same genetic locus as the first marker DNA under the control of the second promoter; and 2) stably integrated into the nuclear genome of the plant's cells.

[0006] The invention further relates to hybrid seeds produced by crossing: 1) the restorer plant, preferably also containing, stably integrated in its nuclear genome, the first marker DNA encoding a protein conferring a resistance to a herbicide on the restorer plant; with 2) a nuclear male- or female-sterile plant which has, stably integrated in its nuclear genome a) the sterility DNA under the control of the sterility promoter and, adjacent to the sterility DNA, preferably within the same genetic locus of the nuclear genome, b) a second marker DNA, encoding a fourth RNA, protein or polypeptide and preferably also conferring a herbicide resistance on the sterile plant, under the control of a third promoter capable of directing expression of the second marker DNA in at least a specific tissue or specific cells in which expression of the second marker DNA renders the plant easily separable or distinguishable from those in which there is not such expression. This invention particularly relates to such hybrid seeds as produced on a commercial scale, preferably in a substantially random population, with increased efficiency of cross-pollination and without the need for extensive hand-labor.

Background of the Invention

[0007] Hybridization of plants is recognized as an important process for producing offspring having a combination

of the desirable traits of the parent plants. The resulting hybrid offspring often have the ability to outperform the parents in different traits, such as in yield, adaptability to environmental changes, and disease resistance. This ability is called "heterosis" or "hybrid vigor". As a result, hybridization has been used extensively for improving major crops, such as corn, sugarbeet and sunflower. For a number of reasons, primarily related to the fact that most plants are capable of undergoing both self-pollination and cross-pollination, the controlled cross-pollination of plants without significant self-pollination, to produce a harvest of hybrid seeds, has been difficult to achieve on a commercial scale.

[0008] In nature, the vast majority of crop plants produce male and female reproductive organs on the same plant, usually in close proximity to one another in the same flower. This favors self-pollination. Some plants, however, are exceptions as a result of the particular morphology of their reproductive organs which favors cross-pollination. These plants produce hybrid offspring with improved vigor and adaptability. One such morphology in Cannabis ssp. (hemp) involves male and female reproductive organs on separate plants. Another such morphology in Zea mays (corn) involves male and female reproductive organs on different parts of the same plant. Another such morphology in Elaeis guineensis (oilpalm) involves male and fertile female gametes which become fertile at different times in the plant's development.

[0009] Some other plant species, such as Ananas comosus (pineapple), favor cross-pollination through the particular physiology of their reproductive organs. Such plants have developed a so-called "selfincompatibility system" whereby the pollen of one plant is not able to fertilize the female gamete of the same plant or of another plant with the same genotype.

[0010] Some other plant species favor cross-pollination by naturally displaying the so-called genomic characteristic of "male-sterility". By this characteristic, the plants' anthers degenerate before pollen, produced by the anthers, reaches maturity. See: "Male-Sterility in Higher Plants", M.L.H. Kaul, 1987, in: Monographs on Theoretical and Applied Genetics 10, Edit. Springer Verlag. Such a natural male-sterility characteristic is believed to result from a wide range of natural mutations, most often involving deficiencies, and this characteristic can not easily be maintained in plant species that predominantly self-pollinate, since under natural conditions, no seeds will be produced.

[0011] Some types of naturally occurring male-sterility are cytoplasmatically encoded, while others are nuclear encoded. One type of male-sterility is the result of a combination of both nuclear encoded male-sterility and' cytoplasmatically encoded male-sterility. The male-sterility inducing nuclear alleles are usually recessive, and only plants that contain the male-sterility cytoplasmic allele and that are homozygous for the male-sterility inducing nuclear allele are phenotypically male-sterile. In this type of plant, corresponding dominant male-fertility inducing alleles or "fertility restorers" produce a male-fertile phenotype. As a result, the male-sterile offspring of this type of plant can be made male-fertile by pollinating the male-sterile plants with pollen containing the fertility restorers . As a result, the offspring of plants of this type are of commercial value where the economic product is seeds (e.g., for plants such as corn, sorghum and sunflower).

[0012] Most of the known naturally occurring male-sterility genes and their corresponding fertility-restorer genes have not been used in breeding or production of new varieties for essentially two reasons: a) insufficient quality of the genes responsible for the male-sterility and restoration characteristics; and b) low cross-pollination capability of the crops in which they occur.

1. The quality of the genes

[0013] To realize the full potential of a male-sterility/ fertility-restorer system, several quality requirements have to be achieved:

a) Stability of the genes encoding the male-sterility under a broad range of different environmental conditions. Most of the currently known systems, whether they are nuclear or cytoplasmatically encoded, do not display sufficient stability. As a consequence of this, under some unpredictable climatological conditions, self-pollination occurs within the plants, and heterogeneous offspring are harvested. According to seed certification requirements, not more than 1% of non-hybrid seed is tolerated for most major field crops.

b) No side effects on the plants. Many cytoplasmic male-sterility genes induce a decrease in plant vigor. This can be tolerated up to a certain level, if the hybrid vigor effect offers a significant improvement of the crop compared to the negative effect. Another side effect which has been observed in crops carrying male-sterility genes consists of an enhanced sensitivity to some plant pathogens (e.g., corn plants carrying T-cytoplasmic male-sterility are highly susceptible to Helminthosporium maydis infections). Restorer genes also often display negative side effects although these are usually not due to the genes themselves but to genes closely linked to the restorer genes. These side effects consist, in most cases, of an increased disease or pest susceptibility or a decreased quality of the crop.

2. Efficiency of cross-pollination

[0014] Reasonably efficient cross-pollination is essential for the production of hybrid seeds at an acceptable cost. For major field crops that are poorly adapted to cross-pollination, it is unrealistic to assure cross-pollination by hand. Therefore, it has been envisaged to sell, as a commercial product, not the F1 hybrid, but the selfed F_2 offspring thereof (e.g., cotton and wheat). The disadvantage of this method lies, however, in the loss of homogeneity and heterosis and the segregation of specific useful gene combinations. To assure high yield of a crop by a farmer, it is advantageous that hybrid crops be fully fertile (with the exception of very efficient cross-pollinating species such as corn and oilseed rape). This is particularly the case with crops that form heavy or sticky pollen which is not easily transported by wind (e.g., cotton), with crops that are not attractive to pollinating insects (e.g., wheat) and with crops which display cleistogamy (e.g., soybean).

Detailed Description of the Invention

[0015] In accordance with this invention, a fertility-restorer plant is produced from a single cell of a plant by transforming the plant cell in a well known manner to stably insert, into the nuclear genome of the cell, the foreign DNA sequence of this invention. The foreign DNA sequence comprises at least one fertility-restorer DNA that is under the control of, and fused at its 5' end to, the first promoter and is fused at its 3' end to suitable transcription termination (or regulation) signals, including a polyadenylation signal. Thereby, the first RNA, protein or polypeptide is produced or overproduced in cells of at least each of the restorer plant's flowers, preferably one or more male or one or more female reproductive organs thereof, and/or seeds and/or embryos, so that when the restorer plant is crossed with a nuclear male-sterile or nuclear female-sterile plant, hybrid male-fertile female-fertile offspring are obtained. The foreign DNA sequence can also comprise at least one first marker DNA that is under the control of, and is fused at its 5' end to, the second promoter and is fused at its 3' end to suitable transcription termination signals, including a polyadenylation signal. The first marker DNA is preferably in the same genetic locus as the fertility-restorer DNA, whereby the third RNA, protein or polypeptide is produced in at least the specific tissue or specific cells of the fertility-restorer plant so that the plant. can be easily distinguished and/or separated from other plants that do not contain the third RNA, protein or polypeptide in the specific tissue or specific cells. This guarantees, with a high degree of certainty, the joint segregation of both the fertility-restorer DNA and the first marker DNA into offspring of the plant.

[0016] The cell of a plant (particularly a plant capable of being infected with Agrobacterium) is preferably transformed in accordance with this invention, using a vector that is a disarmed Ti-plasmid containing the foreign DNA sequence and carried by Agrobacterium. This transformation can be carried out using procedures described, for example, in European patent publications 0,116,718 and 0,270,822. Preferred Ti-plasmid vectors contain the foreign DNA sequence between the border sequences, or at least located to the left of the right border sequence, of the T-DNA of the Ti-plasmid. Of course, other types of vectors can be used to transform the plant cell, using procedures such as direct gene transfer ((as described, for example, in European patent publication 0,223,247), pollen mediated transformation (as described, for example, in European patent publication 0,270,356, PCT publication WO85/01856, and European patent publication 0,275,069), in vitro protoplast transformation (as described for example, in US patent 4,684,611), plant RNA virus-mediated transformation (as described, for example, in European patent publication 0,067,553, and US patent 4,407,956) and liposome-mediated transformation (as described, for example, in US patent 4,536,475).

[0017] Preferably, a fertility-restorer plant of this invention is provided by transforming a plant cell with a disarmed Ti-plasmid vector containing the foreign DNA sequence with a fertility-restorer DNA under the control of a first promoter and optionally a first marker DNA under the control of a second promoter. The marker DNA can be upstream or downstream of the fertility-restorer DNA in the Ti-plasmid vector, but preferably, the two are adjacent to one another and are located between the border sequences or at least located to the left of the right border sequence of the Ti-plasmid vector, so that they are properly transferred together into the nuclear genome of the plant cell. However, if desired, the cell can initially be transformed with the foreign DNA sequence containing the fertility-restorer DNA and the first promoter and can subsequently be transformed with the marker DNA and the second promoter, inserted into the same genetic locus in the cell's nuclear genome as the fertility-restorer DNA, or this transformation can be carried out vice versa. Suitable vectors for this purpose are the same as those discussed above for transforming cells with the foreign DNA sequence. The preferred vector is a disarmed Ti-plasmid vector.

[0018] The selection of the fertility-restorer DNA of this invention is not critical but is dependent on the selection of, and must correspond to, the sterility DNA which is responsible for the male- or female-sterility characteristic to be restored. In particular, the production or overproduction of the first, protein or polypeptide encoded by the fertility-restorer DNA has to neutralize, block, offset, overcome or otherwise prevent the specific activity of the second protein or polypeptide which is a ribonuclease encoded by the sterility DNA in flower cells, preferably cells of at least one male or at least one female reproductive organ, or in seed cells or in embryo cells of the restored plant. Examples of male- and female- sterility DNAs, to which the fertility-restorer DNAs of this invention must correspond, and the action of

which they must counteract, are described in European patent publications 0,344,029 and 0,412,006, respectively, which are incorporated herein by reference. A suitable fertility-restorer DNA can be selected and isolated in a well-known manner to overcome the effects of the sterility DNA in any cell of a flower, particularly a male or female organ, a seed and/or an embryo, in which the sterility promoter causes the sterility DNA to be expressed.

[0019] Preferred examples of fertility-restorer DNAs encode: barstar which neutralizes the activity of barnase (which degrades RNA molecules by hydrolyzing the bond after any guanine residue).

[0020] Still further examples of fertility-restorer DNAs can be combinations of one or more of the different fertility-restorer DNAs cited above.

[0021] By "foreign" with regard to the foreign DNA sequence of this invention is meant that the foreign DNA sequence contains a foreign fertility-restorer DNA and/or a foreign first promoter. By "foreign" with regard to a DNA, such as a fertility-restorer DNA and a first promoter, as well as a first marker DNA, a second promoter and any other DNA in the foreign DNA sequence, is meant that such a DNA is not in the same genomic environment in a plant cell, transformed with such a DNA in accordance with this invention, as is such a DNA when it is naturally found in the cell of the plant, bacteria, animal, fungus, virus, or the like, from which such a DNA originates. This means, for example, that a foreign fertility-restorer DNA or first marker DNA can be: 1) a nuclear DNA in a plant of origin; 2) endogenous to the transformed plant cell (i.e., from a plant of origin with the same genotype as the plant being transformed); and 3) within the same transcriptional unit as its own endogenous promoter and 3' end transcription regulation signals (from the plant of origin) in the foreign DNA sequence of this invention in the transformed plant cell; but 4) inserted in a different place in the nuclear genome of the transformed plant cell than it was in the plant of origin so that it is not surrounded in the transformed plant cell by the genes which surrounded it naturally in the plant of origin. A foreign fertility-restorer or first marker DNA can also, for example, be: 1) a nuclear DNA in a plant of origin; and 2) endogenous to the transformed plant cell; but 3) in the same transcriptional unit as a different (i.e., not its own) endogenous promoter and/or 3' end transcription regulation signals in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. A foreign fertility-restorer or first marker DNA can also, for example, be: 1) a nuclear DNA in a plant of origin; and 2) endogenous to the transformed plant cell; but 3) in the same transcriptional unit as a heterologous promoter and/or 3' end transcription regulation signals in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. A foreign fertility-restorer or first marker DNA can also, for example, be heterologous to the transformed plant cell and in the same transcriptional unit as an endogenous promoter and/or 3' transcription regulation signals (e.g., from the nuclear genome of a plant with the same genotype as the plant being transformed) in a foreign chimaeric DNA sequence of this invention in a transformed plant cell. An example of a foreign fertility-restorer DNA could come from the nuclear genome of a plant with the same genotype as the plant being transformed and encode an inhibitor of a ribonuclease, that is endogenous to the plant being transformed, so that the enzyme is overproduced in transformed cells in order to neutralize the activity of a ribonuclease (i.e., a second protein encoded by a male- or female-sterility DNA) which would significantly disturb adversely the metabolism, functioning and/or development of flower cells, particularly male or female organ cells, or seed cells or embryo cells, in which such an enzyme is expressed. Preferably, each fertility-restorer DNA and first marker DNA is heterologous to the plant cell being transformed.

[0022] By "heterologous" with regard to a DNA, such as a fertility-restorer DNA, a first or third promoter, a first marker DNA and any other DNA in the foreign DNA sequence of this invention, is meant that such a DNA is not naturally found in the nuclear genome of cells of a plant with the same genotype as the plant being transformed. Examples of heterologous DNAs include chloroplast and mitochondrial DNAs obtained from a plant with the same genotype as the plant being transformed, but preferred examples are chloroplast, mitochondrial, and nuclear DNAs from plants having a different genotype than the plant being transformed, DNAs from animal and bacterial genomes, and chromosomal and plasmidial DNAs from fungal and viral genomes.

[0023] By "chimaeric" with regard to the foreign DNA sequence of this invention is meant that at least one of its fertility-restorer DNAs: 1) is not naturally found under the control of its first promoter for the one fertility-restorer DNA; and/or 2) is not naturally found in the same genetic locus as at least one of its first marker DNAs. Examples of foreign chimaeric DNA sequences of this invention comprise: a fertility-restorer DNA of bacterial origin under the control of a first promoter of plant origin; and a fertility-restorer DNA of plant origin under the control of a first promoter of plant origin and in the same genetic locus as a first marker DNA of bacterial origin.

[0024] By "flower" is meant to include the entire shoot axis, sepals, petals, male reproductive organs (or stamens) and/or female reproductive organs (or carpels) whose wholly or partly, retarded or arrested development would prevent the development and/or propagation of viable seeds in the flower or the development and/or propagation of its male gametes; by "male organ" or "male reproductive organ" is meant the entire organ of a flower that is involved in the production of the male gamete, as well as one or more of its individual parts such as its anther, pollen and filament; and by "female organ" or "female reproductive organ" is meant the entire organ of a flower that is involved in the production of the female gamete and/or viable seeds and/or viable embryos, as well as one or more of its individual parts such as its ovary, ovule, style, stigma, corolla, disc, septum, callyx and placenta. By "embryo" is meant to include the entire embryo of a plant, as well as one or more of its individual parts such as its embyo axis and embryo cotyledons.

[0025] So that the fertility-restorer DNA is expressed in at least those specific cells of a fertility-restored plant in which the sterility DNA is expressed, it is preferred that the first promoter, which controls expression of the fertility-restorer DNA, be a promoter capable of directing gene expression in at least the same fertility-restored plant cells (i.e., the specific flower cells, preferably male or female organ cells, or seed cells or embryo cells), in which the sterility DNA is selectively expressed under the control of the sterility promoter. Such a first promoter can be an endogenous promoter or an exogenous promoter and can be from the nuclear genome or from the mitochondrial or chloroplast genome of a plant cell. In any event, the first promoter is foreign to the nuclear genome of the plant cell being transformed. The first promoter can be a constitutive promoter but can also be the same selective promoter as the sterility promoter. Preferably, the first promoter causes the restoration of fertility through the production of at least sufficient amounts of fertility-restoring first RNA, protein or polypeptide selectively in the same specific flower, seed or embryo cells, particularly in the same specific flower cells, as those in which the sterility DNA is expressed.

[0026] The first promoter of this invention can be selected and isolated in a known manner from a plant species, for example as described in: European patent publication 0,344,029 which is incorporated herein by reference and which discloses a male-sterility promoter that directs expression of a sterility DNA selectively in stamen (e.g., anther) cells of a plant and is effective to prevent expression of the sterility DNA in other parts of the plant; and European patent publication 0,412,006 which is also incorporated herein by reference and which discloses a female-sterility promoter that directs expression of a sterility DNA selectively in cells of flowers, particularly cells of a female organ (e.g., pistil), or seed cells or embryo cells of a plant and is effective to prevent expression of the sterility DNA in other parts of the plant. For example, a suitable endogenous organ- or tissue-specific first promoter can be identified and isolated in a plant, by:

1. searching for an mRNA which is only present in the plant during the development of its flowers, seeds or embryos, preferably its anthers, pollen, filaments, ovary, ovule, style, stigma, placenta, calyx, scutellum, septum, seedcoat, endosperm or embryo cotyledons;
2. isolating this specific mRNA;
3. preparing a cDNA from this specific mRNA;
4. using this cDNA as a probe to identify the regions in the plant genome which contain DNA coding for the specific mRNA; and then
5. identifying the portion of the plant genome that is upstream (i.e., 5') from the DNA coding for the specific mRNA and that contains the promoter of this DNA.

[0027] The genes controlled by these first promoters can further be used as probes as in step 4, above. Under hybridizing conditions, such a probe will hybridize to DNA coding for a specific mRNA in a mixture of DNA sequences from the genome of another plant species (Maniatis et al (1982) Molecular Cloning. A Laboratory Manual. Ed. Cold Spring Harbor Laboratory). Thereafter, as in step 5 above, a specific first promoter for another plant species can be identified.

[0028] Examples of male organ-specific first promoters and sterility promoters are: the PTA29 promoter, the PTA26 promoter and the PTA13 promoter, as described in European patent publication 0,344,029, which have been isolated from tobacco and are tapetum-specific promoters; as well as any promoter of a gene encoding a tapetum-specific mRNA hybridizable to the genes TA29, TA26 or TA13 of European patent publication 0,344,029, from which genes the PTA29, PTA26 and PTA13 promoters have been isolated. Examples of female organ-specific first promoters and sterility promoters are: the style and/or stigma-specific promoters, such as PSTMG07, PSTMG08, PSTMG4B12 and PSTMG3C9, and the ovule-specific promoter corresponding to the cDNA clone pMON9608 as described in European patent publication 0,412,006; as well as a promoter of a gene encoding i) a style-stigma specific or ii) an ovule-specific mRNA hybridizable respectively to i) a STMG-type style-stigma specific gene or ii) CDNA clone pMON9608 of European patent publication 0,412,006.

[0029] If more than one nuclear sterility DNA is present in the transgenic sterile plant which is to be crossed with the transgenic fertility-restorer plant of this invention, the restorer plant may need to have inserted into its nuclear genome more than one fertility-restorer DNA of this invention, corresponding in number at least to the number of sterility DNAs in the nuclear genome of the sterile plant. All the fertility-restorer DNAs can be under the control of a single first promoter, but preferably, each fertility-restorer DNA is under the control of its own separate first promoter, which will direct expression of the first RNA, protein or polypeptide at least in those cells where the sterility promoters cause the sterility DNAs to express the second RNA, protein or polypeptide. Each fertility-restorer DNA is adjacent to its first promoter, and all the fertility-restorer DNA(s) and their first promoter(s) are preferably adjacent to one another in the foreign DNA sequences of this invention and in any vectors used to transform plant cells with such foreign DNA sequences. However, it is not necessary that the fertility-restorer DNAs be adjacent to one another in the foreign DNA sequence, and in some cases, they may be inserted into the nuclear genome of the restorer plant through independent transformation events.

[0030] The selection of the first marker DNA of this invention also is not critical. A suitable first marker DNA can be

selected and isolated in a well known manner, so that it encodes the third RNA, protein or polypeptide that allows plants, expressing the first marker DNA, to be easily distinguished and separated from plants not expressing the first marker DNA. In many cases, the first marker DNA encodes the same RNA, protein or polypeptide as the second marker DNA encodes in the nuclear male- or female-sterile plant, the fertility of which is to be restored in accordance with this invention. Examples of the first marker DNAs are the marker DNAs in the nuclear genomes of the nuclear male- and female-sterile plants described in European patent publications 0,344,029 and 0,412,006 which encode proteins or polypeptides that: provide a distinguishable color to plant cells, such as the A1 gene encoding dihydroquercetin-4-reductase (Meyer et al (1987) Nature 330, 677-678) and the glucuronidase gene (Jefferson et al (1988) Proc. Natl. Acad. Sci. USA ("PNAS") 83, 8447); provide a specific morphological characteristic to a plant such as dwarf growth or a different shape of the leaves; confer on a plant stress tolerance, such as is provided by the gene encoding superoxide dismutase as described in European patent application 88402222.9 which is the priority application claimed in European patent publication 0,359,617, confer disease or pest resistance on a plant, such as is provided by a gene encoding a Bacillus thuringiensis endotoxin conferring insect resistance as described in European patent publication 0,193,259; or confer on a plant a bacterial resistance such as is provided by the bacterial peptide described in European patent publication 0,299,828.

[0031] Preferred first marker DNAs encode third proteins or polypeptides inhibiting or neutralizing the activity of herbicides such as: the sfr gene and the sfrv gene encoding enzymes conferring resistance to glutamine synthetase inhibitors such as Bialaphos and phosphinotricine as described in European patent publication 0,242,246; and genes encoding modified target enzymes for certain herbicides that have a lower affinity for the herbicides than naturally produced endogenous enzymes, such as a modified glutamine synthetase as target for phosphinotricine as described in European patent publication 0,240,792 and a modified 5-enolpyruvylshikimate-3 phosphate synthase as a target for glyphosate as described in European patent publication 0,218,571. Other first marker DNAs encode third proteins which neutralize the action of the herbicide bromoxynil (Stalker et al (1988) in: Genetic Improvements of Agriculturally important Crops. Ed: R.T. Fraley, N.M. Frey and J. Schell. Cold Spring Harbor Laboratories) or the herbicide sulfonylurea (Lee et al (1988) EMBO J. 7, 1241-1248) or the herbicide 2,4 D (disclosed at the 2nd International Symposium of Plant Molecular Biology, Jerusalem, 13-18 November 1988).

[0032] The second promoter of this invention, which controls the first marker DNA, can also be selected and isolated in a well known manner so that the first marker DNA is expressed either selectively in one or more specific tissues or specific cells or constitutively in the entire plant, as desired depending on the nature of the third RNA, protein or polypeptide. In many cases, the second promoter is the same as the third promoter which controls the second marker DNA in the male- or female-sterile plant, the fertility of which is to be restored in accordance with this invention. For example, if the first marker DNA encodes an herbicide resistance, it may be useful to have the first marker DNA expressed in all cells of the plant, using a strong constitutive second promoter such as a 35S promoter (Odell et al (1985) Nature 313, 810-812), a 35S'3 promoter (Hull and Howell (1987) Virology 86, 482-493), the promoter of the nopaline synthetase gene ("PNOS") of the Ti-plasmid (Herrera-Estrella (1983) Nature 303, 209-213) or the promoter of the octopine synthase gene ("POCS" [De Greve et al (1982) J. Mol. Appl. Genet. 1 (6), 499-511]). If the first marker DNA encodes a protein conferring disease resistance, it may be useful to have the first marker DNA selectively expressed in wound tissue by using, for example, a second promoter which is a TR promoter such as the TR1' or TR2' promoter of the Ti-plasmid (Velten et al (1984) EMBO J. 3, 2723-2730). If the first marker DNA encodes a herbicide resistance, it may be useful to have the first marker DNA selectively expressed in green tissue by using as the second promoter, for example, the promoter of the gene encoding the small subunit of Rubisco (European patent publication 0,242,246). If the first marker DNA encodes a pigment, it may be useful to select the second promoter so that the first marker DNA is expressed in specific cells, such as petal cells, leaf cells or seed cells, preferably in the outer layer of the seed coat.

[0033] One can identify and isolate in a well known manner a tissue-specific second promoter, suitable for inclusion in the foreign DNA sequence of this invention in a restorer plant or a restored plant of this invention, whereby the plant can be easily distinguished as carrying the first marker DNA under the control of the second promoter. This can be done by:

1. searching for an mRNA which is only present in the plant during the development of a specific tissue, such as its petals, leaves or seeds;
2. isolating this tissue-specific mRNA;
3. preparing a cDNA from this tissue-specific mRNA;
4. using this cDNA as a probe to identify the regions in the plant genome which contain DNA coding for the tissue-specific mRNA; and then
5. identifying the portion of the plant genome that is upstream from the DNA coding for the tissue-specific mRNA and that contains the promoter for said DNA.

[0034] If more than one first marker DNA is present in the foreign DNA sequence of this invention, all the first marker

DNAs can be under the control of a single second promoter, but preferably, each first marker DNA is under the control of its own separate second promoter. More preferably, each first marker DNA is under the control of its own second promoter and encodes a different third RNA, protein or polypeptide, providing different distinguishable characteristics to a transformed plant. In any event, the first marker DNA(s) and second promoter(s) should be adjacent to each other and to the one or more fertility-restorer DNAs contained in the foreign DNA sequence of this invention and in any vector used to transform plant cells with the foreign DNA sequence.

[0035] It is generally preferred that the first RNA, protein or polypeptide, encoded by the fertility-restorer DNA, substantially preventsthe activity of the second RNA, protein or polypeptide, encoded by the sterility DNA, in the cytoplasm or the nucleus of the plant cells in which the sterility DNA is expressed. However, when it is desired to have the first protein or polypeptide and/or the third protein or polypeptide transported from the cytoplasm into chloroplasts or mitochondria of the cells of transformed plants, the foreign DNA sequence can further include an additional foreign DNA encoding a transit peptide. The additional DNA is between the fertility-restorer DNA and the first promoter if the first protein or polypeptide is to be so-transported and is between the first marker DNA and the second promoter if the third protein or polypeptide is to be so-transported. By "transit peptide" is meant a polypeptide fragment which is normally associated with a chloroplast or mitochondrial protein or subunit of the protein and is produced in a cell as a precursor protein encoded by the nuclear DNA of the cell. The transit peptide is responsible for the translocation process of the nuclear-encoded chloroplast or mitochondrial protein or subunit into the chloroplast or the mitochondria, and during such a process, the transit peptide is separated or proteolytically removed from the chloroplast or mitochondrial protein or subunit. One or more of such additional DNAs can be provided in the foreign DNA sequence of this invention for transporting one or more first or third proteins or polypeptides as generally described in European patent publication 0,189,707 and European patent application 88402222.9 (supra) and in: Van den Broeck et al (1985) Nature 313, 358-363; Schatz (1987) Eur. J. of Bioch. 165, 1-6; and Boutry et al (1987) Nature 328, 340-342. An example of a suitable transit peptide for transport into chloroplasts is the transit peptide of the small subunit of the enzyme RUBP carboxylase (European patent publication 0,189,707) and an example of a transit peptide for transport into mitochondria is the transit peptide of the enzyme Mn-superoxide dismutase (European patent publication 0,344,029).

[0036] In the foreign DNA sequence of this invention, 3' transcription termination signals can be selected from among those which are capable of providing correct transcription termination and polyadenylation of mRNA in plant cells. The transcription termination signals can be the natural ones of the foreign gene or DNA to be transcribed or can be foreign or heterologous. Examples of heterologous transcription termination signals are those of the octopine synthase gene (Gielen et al (1984) EMBO J. 3, 835-845) and the T-DNA gene7 (Velten and Schell (1985) Nucleic Acids Research ("NAR") 13, 6981-6998).

[0037] Also in accordance with this invention, a culture of plant cells, containing the foreign DNA sequence of this invention, can be used to regenerate homozygous dominant fertility-restorer plants by performing the necessary transformation: on a haploid cell culture (Chuong and Beversdof (1985) Plant Sci. 39, 219-226) and then doubling the number of chromosomes by well known techniques (e.g., by the use of colchicine); or alternatively, on a diploid cell culture and then culturing anthers of regenerated plants to produce haploid progeny which can afterwards be rendered diploid. See: Plant Tissue and Cell Culture, Plant Biology 3, A.R. Liss, Inc. N.Y. (1987). Thereby, the foreign DNA sequence will be in homozygous form in the nuclear genome of each of the so-transformed plant cells of the culture. This is preferred for a plant cell culture containing a fertility-restorer DNA under the control of a first promoter which directs gene expression at a given stage of development of: i) the plant's male gametes, such as pollen, especially after meiosis, ii) the plant's female gametes, such as ova, especially after meiosis, or iii) cells derived from the male or female gametes, such as seed or embryo cells, so that the fertility-restorer DNA is present and can be expressed in all male 'or female gametes or plant cells derived therefrom.

[0038] Further in accordance with this invention, processes are provided for producing hybrid seeds which can be grown into hybrid fertility-restored plants. One process involves crossing: a nuclear male-sterile female-fertile plant including at least one second marker DNA under the control of at least one third promoter; with a homozygous nuclear male-fertile restorer plant including at least one nuclear male fertility-restorer DNA under the control of at least one first promoter but without a first marker DNA that is the same as the second marker DNA. In this process, the male-sterile and male-fertile plants are sown at random, and after pollination, the selectable marker, encoded by the second marker DNA, is used to eliminate the fertility-restorer plants, ensuring that seed is only harvested on the male-sterile plants. This guarantees that all harvested seeds are both hybrid and fertile. Another process involves crossing: a nuclear male-sterile female-fertile restorer plant including a nuclear first marker DNA under the control of a second promoter and a nuclear female fertility-restorer DNA under the control of a first promoter in a homozygous form; with a nuclear male-fertile female-sterile restorer plant including at least the same nuclear first marker DNA under the control of a second promoter and a nuclear male fertility-restorer DNA under the control of a first promoter in a homozygous form. Both male-sterile and male-fertile parent plants can be grown in a substantially random population, thereby increasing the chances of cross-pollination, without the need for precise planting patterns, and using the characteristic encoded by the first marker DNA, 100% fertile hybrid seeds can be harvested. Preferably in both of these processes,

the first marker DNA is under the control of a constitutive second promoter and encodes a third protein or polypeptide that renders the sterile plant resistant to a particular herbicide. The non-desirable genotypes can then be destroyed before cross-pollination, using the particular herbicide.

[0039] A process in accordance with this invention of crossing: 1) fertility-restorer plants which contain a fertility-restorer DNA stably integrated in their nuclear genome and transmissible throughout generations as a dominant allele in accordance with this invention, with 2) male- or female-sterile plants which contain a sterility DNA, preferably both a sterility DNA and a second marker DNA, stably integrated in their nuclear genome and transmissible throughout generations as dominant alleles in accordance with European patent publications 0,344,029 and 0,412,006, provides an alternative to, and several advantages over, presently used systems for breeding and producing hybrid crops as described below:

1. For crops which do not easily cross-pollinate and for which the seed is the economic harvest and has low multiplication rates, such as cereals (e.g., wheat, barley and oats), rice, cotton, and many legumes (e.g., soybean and pea), the process of this invention offers the possibility to produce 100% hybrid fertile offspring, thereby guaranteeing high seed set and normal yield. An example of a typical strategy for producing hybrid plants, using as parent plants male-sterile and female-sterile parent plants and a restorer for their respective sterilities, may include the following steps (wherein "FH1" stands for female-sterility linked to herbicide resistance 1, "RF" stands for the restorer of the female-sterility, "M1H1" stands for male-sterility 1 linked to herbicide resistance 1, "M2H2" stands for male-sterility 2 linked to herbicide resistance 2, "RM1" stands for restorer of male-sterility 1, "A" stands for female parent lines, and "B" stands for male parent lines):

A. Development of the female parent plant A

1Aa) Transform plant A with a fertility-restorer DNA of this invention that encodes a first RNA, protein or polypeptide (which neutralizes specifically the expression product of the female-sterility DNA in the male parent) and is under the control of a first promoter which directs gene expression in at least the same cells as those in which the female-sterility DNA in the male plant is to be expressed. This gives rise to $A^{RF/rf}$.

1Ab) Self-pollinate $A^{RF/rf}$, giving rise to 25 % $A^{RF/RF}$ plants.

1Ac) Transform $A^{RF/RF}$ with a chimaeric DNA sequence including a "male-sterility DNA 1" under the control of a male organ specific promoter and a marker DNA conferring resistance to a herbicide 1. This gives rise to the male-sterile plant $A^{RF/RF;M1H1/mh}$.

1Ad) Multiply the male-sterile plant by crossing:
$A^{RF/RF;M1H1/mh} \times A^{RF/RF;mh/mh}$
giving an offspring consisting of:

50% $A^{RF/RF;M1H1/mh}$: male-sterile 1, resistant to herbicide 1 and
50% $A^{RF/RF;mh/mh}$: male fertile, herbicide sensitive.

This mixture is sown in successive generations of upscaling of the female parent, and the herbicide 1 is used in alternate rows or blocks of rows to create pure female parent stocks. The rows or blocks of rows where the herbicide is not applied are used as pollen source. Only seed of the herbicide treated rows or blocks of rows is harvested to constitute the next generation.

B. Development of the male parent plant B For the economic production of B, the female-sterile parent line requires the use of two different sterility DNAs. The first one is a female-sterility DNA under the control of a promoter which directs gene expression selectively in cells of the female organ of the plant and linked to a marker DNA conferring resistance to herbicide 1. The second one is a male-sterility DNA (different from male-sterility DNA 1 and called "male-sterility DNA 2"), under the control of a promoter which directs gene expression selectively in male organ cells of the plant and is linked to a second marker DNA conferring resistance to herbicide 2.

1Ba) Transform plant B with a foreign DNA sequence of this invention encoding a first RNA, protein or polypeptide which neutralizes specifically the activity of the male-sterility DNA 1 expressed in the female parent and is under the control of a first promoter which directs gene expression in at least the same male organ cells in which the male-sterility DNA 1 in the female parent plant is expressed. This gives rise to $B^{RM1/rm}$.

1Bb) Self-pollinate $B^{RM1/rm}$, giving rise to 25% $B^{RM1/RM1}$.

1Bc)

(1) Transform $B^{RM1/RM1}$ with a chimaeric DNA sequence including the female-sterility DNA under the control of a promoter which directs gene expression selectively in cells of the female-organ of the plant and a marker DNA conferring resistance to herbicide 1. This gives rise to the female-sterile plant $B^{RM1/RM1;FH1/fh}$.

(2) Transform $B^{RM1/RM1}$ with a chimaeric DNA sequence including the male-sterility DNA 2 under the control of a male organ-specific promoter and a marker DNA conferring herbicide resistance to herbicide 2. This gives rise to the male-sterile plant $B^{RM1/RM1;M2H2/mh}$.

1Bd)

(1) Multiply the male-sterile plant of 1Bc) (2) by crossing:
$B^{RM1/RM1;M2H2/mh}$ X $B^{RM1/RM1;mh/mh}$
giving an offspring consisting of:

50 % $B^{RM1/RM1;M2H2/mh}$: male-sterile,
resistant to herbicide and
50 % $B^{RM1/RM1;mh/mh}$: male fertile, herbicide sensitive.

(2) Multiply the female-sterile plant of 1Bc) (1) by crossing:
$B^{RM1/RM1;M2H2/mh;fh/fh}$ x
$B^{RM1/RM1;mh/mh;FH1/fh}$ which are planted in separate rows, giving rise to the following genotypes in the male-sterile rows:

25 % $B^{RM1/RM1;M2H2/mh;FH1/fh}$: sterile and
resistant to herbicide 1 and 2,
25 % $B^{RM1/RM1;mh/mh;FH1/fh}$: female-sterile and resistant to herbicide 1,
25 % $B^{RM1/RM1;M2H2/mh;fh/fh}$: male-sterile and
resistant to herbicide 2, and
25 % $B^{RM1/RM1;mh/mh;fh/fh}$: /fh: fertile and herbicide sensitive.

1Be) This mixture can be used again as the male parent ($B^{RM1/RM1;mh/mh;FH1/fh}$) in further multiplication crosses, whereby spraying in each generation with herbicide 1 eliminates the female fertile plants and so maintains the male parent line. This mixture will be planted in alternate rows or blocks of rows with the mixture obtained in 1 Bd (1), which mixture will be treated with herbicide 2 to eliminate male fertile plants. Alternatively, the mixture obtained in 1 Bd) (2) can be sown as such and alternate rows can be treated either with herbicide 1 or either with herbicide 2. Under such circumstances, step 1 Bd)(1) is not necessary.

C. Production of hybrid seed AB
Sow at random the mixtures obtained in the steps 1Ad) and 1Be). Before cross-pollination occurs, spray with herbicide 1 in order to eliminate all undesirable genotypes. Cross pollination occurs with:
$A^{RF/RF;\ rm/rm;M1H1/mh:fh/fh}$ X $B^{RM1/RM1;\ rf/rf;\ mh/mh;FH1/fh}$, giving rise to:

25 % $AB^{RF/rf;M1H1/mh:rm/RM1:FH1/fh}$
25 % $AB^{RF/rf;M1H1/mh;rm/RM1;fh/fh}$
25 % $AB^{RF/rf;mh/mh;rm/RM1;FH1/fh}$
25 % $AB^{RF/rf;mh/mh;rm/RM1;fh/fh}$

constituting 100 % fertile hybrid seed.

2. Depending on the special characteristics of the crop which is bred, the foregoing general strategy can be simplified. Such special characteristics include:

(2.1) If the crop undergoes a reasonable or good cross-pollination by insects, the relative proportion of parent line B in the mixture can be lowered without affecting the yield of the crop (e.g., cotton, a legume such as Pisum, alfalfa, oilseed rape and corn). Alternatively, a much simplified breeding scheme can be used for a crop involving a female parent which has been rendered male-sterile and herbicide resistant and a male parent carrying the fertility-restorer gene for the male-sterility.
This would permit the following strategy:

Cross: $A^{MH/mh}$ x $B^{RM/RM}$ sown at random or in rows for crops which do not flower synchronously.
Treat with herbicide after pollination when sown at random.
Yielding: 50 % $AB^{MH/mh;RM/rm}$ and 50 % $AB^{mh/mh;RM/rm}$, constituting 100 % fertile hybrid offspring.
(2.2) In case F2 offspring represent the commercial seed product (e.g. cotton), the following variant strategy can be used:

a) Produce by transformation male-sterile plants of parent line A, giving $A^{M/m;r/r}$;
b) Produce by 2 independent transformation events fertility-restorer plants carrying into two independent genetic loci of its nuclear genome the fertility-restorer gene the product of which neutralizes specifically the activity the male-sterility gene in the male-sterile plant of a) and obtain by self-pollination both restorer genes in a homozygous form, giving $B^{m/m;R1/R1;R2/R2}$;
c) Cross $A^{M/m;r/r}$ x $B^{m/m;R1/R1;R2/R2}$
yielding 50 % $AB^{M/m;R1/r;R2/r}$ and 50 % $AB^{m/m;R1/r;R2/r}$
constituting 100 % hybrid fertile offspring; and
d) Self-pollinate the mixture obtained in c).
Half of the offspring are as shown in Table 1, below, only 1 of a total of 64 plants being male-sterile (indicated by an * in Table 1), and all the others being fertile. This result makes this process economically valuable.

Table 1

AB\AB	sR1R2	sR1r2	sr1R2	sr1r2
SR1R2	sR1R2/SR1R2	sR1r2/SR1R2	sr1R2/SR1R2	sr1r2/SR1R2
SR1r2	sR1R2/Sr1R2	sR1r2/SR1r2	sr1R2/SR1r2	sr1r2/SR1r2
Sr1R2	sR1R2/Sr1R2	sR1r2/Sr1R2	sr1R2/Sr1R2	sr1r2/Sr1R2
Sr1r2	sR1R2/Sr1r2	sR1r2/Sr1r2	sr1R2/Sr1r2	sr1r2/Sr1r2*
sR1R2	sR1R2/sR1R2	sR1r2/sR1R2	sr1R2/sR1R2	sr1r2/sR1R2
sR1r2	sR1R2/sR1r2	sR1r2/sR1r2	sr1R2/sR1r2	sr1r2/sR1r2
sr1R2	sR1R2/sr1R2	sR1r2/sr1R2	sr1R2/sr1R2	sr1r2/sr1R2
sr1r2	sR1R2/sr1r2	sR1r2/sr1r2	sr1R2/sr1r2	sr1r2/sr1r2

(2.3) If the male-sterility DNA 2 is linked to another marker DNA than the one encoding resistance to herbicide 2, e.g. a color gene, the plants carrying this male-sterility DNA could be easily eliminated without damage to the other plants. Alternatively, the male-sterility DNA 2 could be introduced without any selectable marker DNA. Eliminating plants carrying the male-sterility DNA 2 could be done through manual selection, which needs only to be done on a small scale (See (1) Bd), above).
(2.4) If the tissue of the parent plants to be transformed is constituted of haploid material, this would reduce considerably the subsequent breeding, presenting the dominant genes encoding sterility in a homozygous form.
(2.5) If the value of the seed, or the cost of hand labor allows manual elimination of unwanted genotypes, at least up to the last stages before the hybrid production, the general system could also be simplified.

3. Another example of a breeding strategy--using male- and female-sterility combined with the fertility restorer system of this invention--may include the following steps:
3A. Development of the female parent line A

3Aa) Transform line A with a foreign DNA sequence including a fertility-restorer DNA of this invention which: encodes a first RNA, protein or polypeptide that neutralizes specifically the activity of the product of a female-sterility DNA expressed in the male parent; is under the control of a first promoter that directs expression of the fertility-restorer DNA in at least the same female organ cells as those in which the female-sterility DNA of the male parent is expressed; and is adjacent to a first marker DNA encoding resistance to herbicide 2. This gives rise to $A^{RFH2/rfh}$. Transform also, in parallel, line A with a DNA sequence including a male-sterility DNA which: is under the control of a male organ-specific promoter; and is adjacent to a second marker DNA encoding a different herbicide resistance (i.e., to herbicide 1) from the one encoded by the first marker DNA. This gives rise to $A^{MH1/mh}$
3Ab) Cross $A^{RFH2/rfh}$ X $A^{MH1/mh}$, giving rise to

25% $A^{RFH2/rfh;MH1/mh}$
25% $A^{RFH2/rfh;mh/mh}$
25% $A^{rfh/rfh;MH1/mh}$
25% $A^{rfh/rfh;mh/mh}$.
Spray with herbicides 1 and 2, selecting
$A^{RFH2/rfh;MH1/mh}$.

3Ac) Self-pollinate $A^{RFH2/rfh}$ X $A^{RFH2/rfh}$,
giving rise to 25% $A^{RFH2/RFH2}$, which can be maintained by self-pollination.
3Ad) Cross $A^{RFH2/RFH2;mh/mh}$ X $A^{RFH2/rfh;\ MH1/mh}$.
This gives rise to:

25% $A^{RFH2/RFH2;\ MH1/mh}$
25% $A^{RFH2/RFH2;\ mh/mh}$
25% $A^{RFH2/rfh;\ MH1/mh}$
25% $A^{RFH2/rfh;\ mh/mh}$

whereby the male-sterile plants, having the fertility-restorer DNA in homozygous form, can be selected by spraying with herbicide 1 and by test-crossing with parental A line.
3Ae) Maintain the female parent line A by crossing:
$A^{RFH2/RFH2;MH1/mh}$ x $A^{RFH2/RFH2;mh/mh}$.

B. Development of the male parent line B

3Ba) Transform line B with a foreign DNA sequence including a fertility-restorer DNA of this invention which: encodes a first RNA, protein or polypeptide that neutralizes specifically the activity of the product of a male-sterility DNA expressed in the female parent; is under the control of a first promoter that directs expression of the fertility-restorer DNA in at least the same male organ cells as those in which the male-sterility DNA is expressed; and is adjacent to a first marker DNA encoding resistance to herbicide 2. This gives rise to $B^{RMH2/rmh}$.
Transform, in parallel, also line B with a DNA sequence including a female-sterility DNA which: is under the control of a female organ-specific promoter; and is adjacent to a second marker DNA encoding resistance to herbicide 1. This gives rise to $B^{FH1/fh}$.
Bb) Cross $B^{RMH2/rmh;fh/fh}$ X $B^{rmh/rmh;FH1/fh}$, giving rise to:

25% $B^{RMH2/rmh;\ FH1/fh}$
25% $B^{RMH2/rmh;\ fh/fh}$
25% $B^{rmh/rmh;FH1/fh}$
25% $B^{rmh/rmh;\ fh/fh}$.
Isolate $B^{RMH2/rmh;FH1/fh}$ by spraying with herbicides 1 and 2.

3Bc) Self-pollinate $B^{RMH2/rmh}$ X $B^{RMH2/rmh}$,
giving rise to:
25% $B^{RMH2/RMH2}$ which can be maintained through self-pollination.
3Bd) Cross $B^{RMH2/RMH2}$ X $B^{RMH2/rmh;FH1/fh}$
giving rise to:

25% $B^{RMH2/RMH2;FH1/fh}$
25% $B^{RMH2/RMH2;fh/fh}$
25% $B^{RMH2/rmh;FH1/fh}$
25% $B^{RMH2/rmh;fh/fh}$

whereby the female-sterile plants having the fertility-restorer DNA in homozygous form are selected by spraying with herbicide 1 and by test-crossing with parental B line.
3Be) Maintain the male parent line B by crossing: $B^{RMH2/RMH2;fh/fh}$ X $B^{RMH2/RMH2;FH1/fh}$.

C. Alternative procedure for development of male or female parent plant (A or B are both designated by C)

3Ca) Transform line C with a foreign DNA sequence including a fertility-restorer DNA of this invention which: encodes a first RNA, protein or polypeptide that neutralizes specifically the activity of the product of a sterility DNA expressed in the other parent; is under the control of a first promoter that directs expression of the fertility restorer DNA in at least the cells in which the sterility DNA of the other parent is expressed; and is adjacent to a first marker DNA encoding resistance to herbicide 2. This gives rise to $C^{RH2/rh}$.

3Cb) Self-pollinate $C^{RH2/rh}$ X $C^{RH2/rh}$, producing 25% $C^{RH2/RH2}$ which can be maintained through self-pollination.

3Cc) Transform $C^{RH2/RH2}$ with a DNA sequence including a sterility DNA which is: under the control of a male or female organ-specific promoter and adjacent to a second marker DNA encoding resistance to herbicide 1. This gives rise to $C^{RH2/RH2;SH1/sh}$ (wherein "S" stands for male- or female-sterility).

3Cd) Maintain line C by the following cross: $C^{RH2/RH2;SH1/sh}$ X $C^{RH2/RH2;sh/sh}$.

3D Production of hybrid seed AB

Sow at random the mixtures obtained in steps 3Ae) and 3Be) or the mixture obtained in step 3Cd). Before cross-pollination occurs, spray with herbicides 1 and 2 in order to eliminate all undesirable genotypes. This leads to the following cross:

$A^{RFH2/RFH2;rm/rm;MH1/mh;fh/fh}$ X

$B^{RMH2/RMH2;rf/rf;FH1/fh;mh/mh}$.

This gives rise to the following offspring:

25% $AB^{RFH2/rf;RMH2/rm;MH1/mh;FH1/fh}$
25% $AB^{RFH2/rf;RMH2/rm;MH1/mh;fh/fh}$
25% $AB^{RFH2/rf;RMH2/rm;mh/mh;FH1/fh}$
25% $AB^{RFH2/rf;RMH2/rm;mh/mh;fh/fh}$

consisting of 100% hybrid fertile seed.

4. Other advantages of the fertility-restorer system of this invention, combined with the male- or female-sterility systems described in European patent publications 0,344,029 and 0,412,006, compared to earlier systems, include:

a) A fool-proof production scheme, with several well distinguishable and selectable markers to control quality;
b) A considerable reduction in complexity at the level of the final seed multiplier, which is essential for reliable production and reduced production costs; and
c) Reduction of the time necessary for the production of a commercial hybrid seed.

[0040] The following Examples illustrate the invention. The figures referred to in the Examples are as follows:

Fig. 1 shows a map of the vector pTVE74 of Example 1.
Fig. 2 shows the DNA sequence of the barstar gene, used in Example 1, and indicates the mutated sequence of its ClaI site.

Unless otherwise stated in the Examples, all procedures for making and manipulating recombinant DNA were carried out by the standardized procedures described in Maniatis et al, Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory (1982). The following plasmids and vectors, used in the Examples, have been deposited in the Deutsche Sammlung Für Mikroorganismen und Zellculturen ("DSM"), Mascheroder Weg 1B, D-3300 Braunschweig, Federal Republic of Germany under the provisions of the Budapest Treaty:

Plasmid or vector	DSM Accession No.	Date
pMB3	4470	21 Mar. 1988
pGSC1700	4469	21 Mar. 1988

Example 1 - Construction of a chimaeric DNA sequence of PTA29 and a barstar gene

[0041] A plasmid named "pTVE74", shown in Fig. 1, is constructed by assembling the following well known DNA fragments with the PTA29 promoter:

1. a vector fragment, including T-DNA border sequences, derived from pGSC1700 (Cornelissen and Vandewiele

(1989) NAR 17 (1) 19-29) in which the β-lactamase gene has been deleted; located between the border sequences are the following DNA fragments 2 and 3;

2. a chimaeric sequence containing the promoter cassette PTA29 from European patent publication 0,344,029, fused in frame at the ATG initiation codon with a Bacillus amyloliquefaciens gene encoding barstar, which is the cellular inhibitor of the extracellular ribonuclease, Barnase (Hartley et al (1972) Preparative Biochemistry 2 (3) 243-250; Hartley and Smeaton (1973) J. Biol. Chem. 248 (16), 5624-5626); the following steps are carried out:

a) The nucleotide sequence GCAC, at positions 7 to 10 up-stream of the first ATG codon, is mutated into nucleotide sequence ATCG, in order to obtain a suitable ClaI cloning site at the first methionine codon of the coding sequence (see Fig.2); this is accomplished using site directed mutagenesis (European patent application 87402348.4 which is the priority application claimed in European patent publication 0,319,353) and yields pMc5-TPBSC; the ClaI protruding ends are digested by the enzyme, SI, and the barstar gene is isolated as a ClaI-HindIII fragment of 330 nucleotides (Fig. 2); and

b) The SI-treated ClaI-HindIII fragment of pMc5-TPBSC is fused with the SI-treated NcoI-HindIII fragment of pMB3 (European Patent publication 0,344,029) and with a restriction fragment containing the 3' end signals of the nopaline synthetase ("NOS") gene for transcription termination and polyadenylation (An et al (1985) EMBO J. 4 (2), 277); and

3. a chimaeric sequence containing an Arabidopsis Rubisco SSU promoter ("PSSU" or "PSSUARA"), a neo gene encoding, kanamycin resistance (European patent publication 0,242,246) and the 3' end signals of the octopine synthase ("OCS") gene (Dhaese et al (1983) EMBO J. 2, 419).

[0042] pTVE74 is a binary type T-DNA vector containing, within the T-DNA border sequences, three chimaeric sequences: PNOS-neo and PSSU-sfr which comprise first marker DNAs under the control of their own second promoters; and PTA29-barstar in which barstar is a fertility-restorer DNA whose expression under the control of the tapetum-specific PTA29 first promoter will neutralize, in tapetum cells of an otherwise male-sterile plant, the activity of Barnase encoded by a sterility DNA under the control of a tapetum-specific sterility promoter as described in European patent publication 0,344,029.

Example 2 - Introduction of the chimaeric DNA sequence of Example 1 into tobacco and oilseed rape

[0043] A recombinant Agrobacterium strain is constructed by mobilizing pTVE74 (from Example 1) from E. coli into Agrobacterium tumefaciens C58C1 Rif^R containing pMP90 (Koncz and Schell (1986) Mol. Gen. Genetics 204, 383-396). The resulting Agrobacterium strain harboring pMP90 and pTVE74 is used to transform tobacco leaf discs (N. tabacum Petite Havane SR1) using standard procedures as described, for example, in European patent publication 0,242,246 and to transform oilseed rape (Brassica napus) according to the procedure of Lloyd et al (1986) Science 234, 464-466 and Klimaszewska et al (1985) Plant Cell Tissue Organ Culture 4, 183-197. Carbenicillin is used to kill the Agrobacterium strains after infection.

[0044] Transformed calli are selected on substrate containing 100 ug/ml kanamycin, and resistant calli are regenerated into plants. After induction of shoots and roots, the transformants are transferred to the greenhouse and grown until they flower. The flowers are examined, and they exhibit a fully natural morphology. Pollens of these flowers are used to pollinate the nuclear male-sterile tobacco and oilseed rape plants containing the Barnase gene as a sterility DNA, under the control of the tapetum cell-specific PTA29 sterility promoter, described in Example 13 of European patent publication 0,344,029. Offspring of these pollinated male-sterile plants are analyzed, and 75% of their flowers do not exhibit a male-sterility phenotype (i.e., absence of a normal tapetum layer in the stamens of their flowers).

[0045] Needless to say, this invention is not limited to the transformation of any specific plant(s). The invention relates to any plant, the nuclear genome of which can be transformed with a fertility-restorer DNA under the control of a first promoter that can direct expression of the fertility-restorer DNA selectively in at least cells of the plant's flowers, particularly at least one male or at least one female organ thereof, and/or seeds and/or embryos, whereby the plant can be both self-pollinated and cross-pollinated. For example, this invention relates to plants such as corn, oilseed rape, wheat, rice, sunflower, sugarbeet, tomato, lettuce, peppers, sorghum, soybean, pea, alfalfa, grasses, clovers, carrot, cabbages, leek, onion, tobacco, petunia, cacao and citrus trees.

[0046] Also, this invention is not limited to the specific plasmids and vectors described in the foregoing Examples, but rather encompasses any plasmids and vectors containing the fertility-restorer DNA under the control of the first promoter.

[0047] Furthermore, this invention is not limited to the specific first promoters described in the foregoing Examples, such as the PTA29 promoter, but rather encompasses any DNA sequence encoding a first promoter capable of directing expression of a fertility-restorer DNA at least in cells of a plant's flowers, seeds and/or embryos, where expression of

a sterility DNA would otherwise cause the plant to be male- or female-sterile. In this regard, the first promoter of this invention encompasses: the promoters described in European patent publication 0,344,029 for use in controlling the expression of a sterility DNA selectively in stamen cells of a plant to be rendered male-sterile; and the promoters described in European patent publication 0,412,006 for use in controlling the expression of a sterility DNA selectively in cells of flowers, seeds or embryos of a plant to be rendered female-sterile. Alternatively, the first promoter can be a constitutive promoter for the plant, provided the first RNA, protein or polypeptide does not significantly disturb adversely the functioning, metabolism or development of cells in which it is expressed in the absence of expression of the sterility DNA.

[0048] In addition, this invention is not limited to the specific fertility-restorer DNAs described in the foregoing Examples but rather encompasses any DNA sequence encoding a first protein or polypeptide which, in a fertility-restored plant, neutralizes, blocks, offsets, overcomes or otherwise prevents the activity of the second protein or polypeptide which is a ribonuclease that is encoded by the sterility-DNA under the control of the sterility promoter and that would otherwise significantly disturb adversely the metabolism, functioning and/or development of cells of flowers, seeds or embryos of the plant.

[0049] Also, this invention is not limited to the specific first marker DNAs described in the foregoing Examples but rather encompasses any DNA sequence encoding a third RNA, protein or polypeptide which confers on at least a specific plant tissue or specific plant cells, in which such DNA sequence is expressed, a distinctive trait compared to such a specific plant tissue or specific plant cells in which such DNA sequence is not expressed.

Claims

1. A recombinant DNA comprising a first chimeric DNA which comprises :

   a) a restorer DNA encoding a protein which is an inhibitor of a ribonuclease, and,
   b) a first promoter which directs expression at least in specific cells of a flower, a seed and/or an embryo of a plant,

   and wherein said restorer DNA is in the same transcriptional unit as, and under the control of, said first promoter.

2. The recombinant DNA of claim 1 in which said inhibitor is capable of neutralizing the activity of the extracellular ribonuclease barnase of Bacillus amyloliquefaciens.

3. The recombinant DNA of claim 2 in which said inhibitor is barstar with an amino acid sequence as encoded by the coding sequence starting at nucleotide position 11 in Figure 2.

4. The recombinant DNA of claim 3 in which said restorer DNA comprises the coding sequence starting at nucleotide position 11 in Figure 2.

5. The recombinant DNA of claim 4 in which said restorer DNA is the ClaI-HindIII fragment of Fig. 2.

6. The recombinant DNA of any one of claims 1 or 5, which also comprises a second chimric DNA, comprising:

   (c) a marker DNA encoding a marker RNA, protein or polypeptide which, when present at least in a specific tissue or in at least specific cells of a plant, renders said plant easily separable from other plants which do not contain said marker RNA, protein or polypeptide in said specific tissue or specific cells; and
   (d) a second promoter capable of directing expression of said marker DNA at least in said specific tissue or specific cells; said marker DNA being in the same transcriptional unit as, and under the control of, said second promoter.

7. The recombinant DNA of claim 6 in which said marker DNA encodes a protein or polypeptide conferring a color to at least said specific tissue or specific cells; or encodes a protein or polypeptide conferring on said plant a stress tolerance, a disease or pest resistance or a bacterial resistance.

8. The recombinant DNA of claim 7 wherein said marker DNA encodes a *Bacillus thuringiensis* endotoxin that confers insect resistance, or encodes a bactericidal peptide that confers a bacterial resistance.

9. The recombinant DNA of claim 6 wherein said marker DNA encodes a modified target enzyme for a herbicide

having lower affinity for the herbicide than the unmodified target enzyme.

10. The recombinant DNA of claim 9 wherein said marker DNA encodes a protein or polypeptide which is selected from the group of a modified 5-enolpyruvylshikimate-3 phosphate synthase as a target for the herbicide glyphosate and a modified glutamine synthetase as a target for a glutamine synthetase inhibitor, including phosphinothricin.

11. The recombinant DNA of claim 6 wherein said marker DNA encodes a protein or polypeptide that inhibits or neutralizes the activity of a herbicide.

12. The recombinant DNA of claim 11 wherein said marker DNA encodes a protein or polypeptide conferring resistance to a glutamine synthetase inhibitor, including phosphinothricin.

13. The recombinant DNA of claim 12 wherein said marker DNA is a sfr or sfrv gene.

14. The recombinant DNA of any one of claims 6 to 13 wherein said second promoter is a constitutive promoter, a wound-inducible promoter, a promoter which directs expression selectively in plant tissue having photosynthetic activity, or a promoter which directs gene expression selectively in leaf cells, petal cells or seed cells.

15. The recombinant DNA of claim 14 wherein said second promoter is a 35S promoter, a 35S'3 promoter, a Pnos promoter, a TR1' or TR2' promoter, or a SSU promoter.

16. The recombinant DNA of any one of claims 1 to 15 which also comprises:

(e) a first DNA encoding a transit peptide capable of transporting said inhibitor into a chloroplast or mitochondria of said stamen cells; said first DNA being in the same transcriptional unit as said restorer DNA and said first promoter and between said restorer DNA and said first promoter; and/or
(f) a second DNA encoding a transit peptide capable of transporting said marker protein or polypeptide into a chloroplast or mitochondria of at least said specific tissue or specific cells; said second DNA being in the same transcriptional unit as said marker DNA and said second promoter and between said marker DNA and said second promoter.

17. The recombinant DNA of any one of claims 1 to 16 in which said first promoter is a constitutive promoter.

18. The recombinant DNA of any one of claims 1 to 16 in which said first promoter directs expression at least in cells of the male organ of a plant.

19. The recombinant DNA of claim 18 in which said first promoter directs expression selectively in stamen cells of a plant.

20. The recombinant DNA of claims 18 or 19 in which said first promoter directs expression in one or more types of stamen cells selected from the group of anther, pollen, and filament cells.

21. The recombinant DNA of claim 20 in which said first promoter directs expression in anther cells.

22. The recombinant DNA of claim 21 in which said first promoter is a promoter from an endogenous plant gene selected from the group of the TA29 gene from tobacco, the TA26 gene from tobacco, the TA13 gene from tobacco, a gene encoding a mRNA hybridizable to said TA29 gene, a gene encoding a mRNA hybridizable to said TA13 gene, and a gene encoding a mRNA hybridizable to said TA26 gene.

23. The recombinant DNA of claim 22 in which said first promoter is the TA29 promoter contained in the NcoI-HindIII fragment of plasmid pMB3, DSM 4470.

24. The recombinant DNA of any one of claims 1 to 16 in which said first promoter directs expression at least in cells of the female organ of a plant.

25. The recombinant DNA of claim 24 in which said first promoter directs expression selectively in cells of the female organs of a plant.

26. The recombinant DNA of claims 24 or 25 in which said first promoter directs expression in one or more types of cells of the female organ selected from the group of ovary, ovule, style, stigma, or septum cells.

27. The recombinant DNA of claim 26 in which said first promoter is a promoter that directs expression in style and/or stigma cells, such as the promoter of the endogenous STGM4B12 gene of tobacco, the endogenous STGM3C9 gene of tobacco, the promoter of the endogenous STGM07 gene from tobacco, or the promoter of the endogenous STGM08 gene from tobacco.

28. The recombinant DNA of any one of claims 1 to 27 which is part of the nuclear genome of a cell of a plant or of a seed.

29. The recombinant DNA of claim 28 which also contains a DNA encoding said ribonuclease.

30. A cell of a plant which contains the recombinant DNA of any one of claims 1 to 29.

31. A cell of claim 30 which can be regenerated into a plant.

32. A plant which contains the DNA of any one of claims 1 to 29.

33. A plant which contains the recombinant DNA of any one of claims 1 to 29 in all of its cells.

34. A seed of a plant wherein said seed contains the recombinant DNA of any one of claims 1 to 29.

35. The seed of claim 34 which is a hybrid seed.

36. A plant which contains integrated into the nuclear DNA of all of its cells, the recombinant DNA of any one of claims 17 to 23 and which is capable, when crossed to a second plant which contains a second recombinant DNA comprising a sterility DNA encoding said ribonuclease under the control of a sterility promoter which directs expression selectively in specific stamen cells of said second plant and which is male-sterile due to the selective production of said ribonuclease in said specific stamen cells, of producing male fertile progeny plants that produce said ribonuclease and said inhibitor of said ribonuclease in said specific stamen cells.

37. The plant of claim 36 which is homozygous for said recombinant DNA.

38. A plant which contains integrated into the nuclear DNA of all of its cells,

a) the recombinant DNA of any one of claims 17 to 23, and,
b) a second recombinant DNA comprising a sterility DNA encoding said ribonuclease under control of a sterility promoter which directs expression selectively in specific stamen cells of said plant,

and which is male fertile due to the neutralizing of the activity of said ribonuclease in said specific stamen cells by said inhibitor produced by expression of said restorer DNA at least in said specific stamen cells.

39. The plant of claim 38 which is a hybrid plant.

40. The plant of any one of claims 36 to 39 in which said ribonuclease is a barnase and said restorer DNA encodes barstar.

41. The plant of any one of claim 36 to 40 in which said sterility promoter directs expression in one or more types of stamen cells selected from the group of anther, pollen, and filament cells.

42. The plant of claim 41 in which said sterility promoter directs expression in one or more types of stamen cells selected from the group of tapetum and anther epidermal cells.

43. The plant of claim 42 in which said sterility promoter is a promoter from an endogenous plant gene selected from the group of the TA29 gene from tobacco, the TA26 gene from tobacco, the TA13 gene from tobacco, a gene encoding a mRNA hybridizable to said TA29 gene, a gene encoding a mRNA hybridizable to said TA13 gene, and a a gene encoding a mRNA hybridizable to said TA26 gene.

44. The plant of claim 43 in which said sterility promoter is the TA29 promoter contained in the NcoI-HindIII fragment of plasmid pMB3, DSM 4470.

45. The plant of any one of claims 36 to 44 in which said first promoter and said sterility promoter are the same.

46. A pair of parent plants for producing seeds comprising:

(a) a male-sterile parent plant which contains incorporated in the nuclear genome of all of its cells, a sterility DNA encoding said ribonuclease under the control of a sterility promoter which directs expression of said sterility DNA selectively in specific stamen cells of said plant, and,
(b) a male-fertile parent plant which contains incorporated into the nuclear DNA of all of its cells, the recombinant DNA of any one of claims 17 to 23 wherein said first promoter directs expression at least in the same specific stamen cells as said sterility promoter;

and whereby said male-sterile and male-fertile plant can be crossed to produce male-fertile progeny comprising said recombinant DNA and said sterility DNA under control of said sterility promoter.

47. The pair of claim 46 in which said ribonuclease is barnase and said restorer DNA encodes barstar.

48. The pair of claim 46 or 47 in which said sterility promoter directs expression in one or more types of stamen cells selected from the group of anther, pollen, and filament cells.

49. The pair of claim 48 in which said sterility promoter directs expression in one or more types of stamen cells selected from the group of tapetum and anther epidermal cells.

50. The pair of claim 49 in which said sterility promoter is a promoter from an endogenous plant gene selected from the group of the TA29 gene from tobacco, the TA26 gene from tobacco, the TA13 gene from tobacco, a gene encoding a mRNA hybridizable to said TA29 gene, a gene encoding a mRNA hybridizable to said TA13 gene, and a a gene encoding a mRNA hybridizable to said TA26 gene.

51. The pair of claim 50 in which said sterility promoter is the TA29 promoter contained in the NcoI-HindIII fragment of plasmid pMB3, DSM 4470.

52. The pair of any one of claims 46 to 51 in which said first promoter and said sterility promoter are the same.

53. The pair of any one of claims 46 to 52 in which said male-fertile parent plant is homozygous for said recombinant DNA.

54. The pair of any one of claims 46 to 53 for producing hybrid seeds.

55. A process for producing a transgenic plant, or reproduction material or progeny plants thereof, which comprises the steps of:

a) transforming a starting cell of a plant with the recombinant DNA of any one of claims 1 to 27 to produce a transformed plant cell which contains said recombinant DNA stably integrated into its nuclear DNA;
b) regenerating said transgenic plant from said transformed cell, and optionally,
c) propagating said transgenic plant to obtain said reproduction material or progeny plants which contain said recombinant DNA.

56. A plant whic contains integrated into the nuclear DNA of all of its ceils, the recombinant DNA of claim 17 or of any one of claims 24 to 27 and which is capable, when crossed to a second plant which contains a second recombinant DNA comprising a sterility DNA encoding said ribonuclease under control of a sterility promoter which directs expression selectively in specific cells of the female organ of said second plant and which is female-sterile due to the selective production of said ribonuclease in said specific cells, of producing female fertile progeny plants that produce said ribonuclease and said inhibitor of said ribonuclease in said specific cells.

57. The plant of claim 56 which is homozygous for said recombinant DNA.

58. A plant which contains integrated into the nuclear DNA of all of its cells,

a) the recombinant DNA of claim 17 or of any one of claims 24 to 27, and,
b) a second recombinant DNA comprising a sterility DNA encoding said ribonuclease under control of a sterility promoter which directs expression selectively in specific cells of the female organ of said plant,

and which is female fertile due to the neutralizing of the activity of said ribonuclease in said specific cells by said inhibitor produced by expression of said restorer DNA at least in said specific cells.

59. The plant of claim 58 which is a hybrid plant.

60. The plant of any one of claims 56 to 59 in which said ribonuclease is barnase and said restorer DNA encodes barstar.

61. The plant of any one of claim 56 to 60 in which said sterility promoter directs expression in one or more types of cells selected from the group of ovary, ovule, style, stigma and septum cells.

62. The plant of claim 61 in which said first promoter is a promoter that directs expression in style and/or stigma cells, such as the promoter of the endogenous STGM4B12 gene of tobacco, the endogenous STGM3C9 gene of tobacco, the promoter of the endogenous STGM07 gene from tobacco, or the promoter of the endogenous STGM08 gene from tobacco.

63. The plant of any one of claims 56 to 62 in which said first promoter and said sterility promoter are the same.

64. A pair of parent plants for producing seeds comprising:

(a) a female-sterile parent plant which contains incorporated in the nuclear genome of all of its cells, a sterility DNA encoding said ribonuclease under the control of a sterility promoter which directs expression of said sterility DNA selectively in specific cells of the female organ of said plant, and,
(b) a female-fertile parent plant which contains incorporated into the nuclear DNA of all of its cells, the recombinant DNA of claim 17 or of any one of claims 24 to 27 wherein said first promoter directs expression at least in the same specific cells of the female organ as said sterility promoter;

and whereby said female-sterile and female-fertile plant can be crossed to produce female-fertile progeny comprising said recombinant DNA and said sterility DNA under control of said sterility promoter.

65. The pair of claim 64 in which said ribonuclease is barnase and said restorer DNA encodes barstar.

66. The pair of claim 64 or 65 in which said sterility promoter directs expression in one or more types of cells selected from the group of ovary, ovule, style, stigma and septum cells.

67. The pair of claim 66 in which said sterility promoter is a promoter that directs expression in style and/or stigma cells, such as the promoter of the endogenous STGM4B12 gene of tobacco, the endogenous STGM3C9 gene of tobacco, the promoter of the endogenous STGM07 gene from tobacco, or the promoter of the endogenous STGM08 gene from tobacco.

68. The pair of any one of claims 64 to 67 in which said first promoter and said sterility promoter are the same.

69. The pair of any one of claims 64 to 68 in which said female-fertile parent plant is homozygous for said recombinant DNA.

70. The pair of any one of claims 64 to 69 for producing hybrid seeds.

71. The use of the recombinant DNA of any one of claims 1 to 28 to produce an inhibitor of a ribonuclease in at least specific cells of a flower, a seed and/or an embryo of a plant.

72. The use of claim 71 to neutralize the activity of said ribonuclease produced in said at least specific cells.

Patentansprüche

1. Rekombinante DNA, umfassend eine erste chimäre DNA, die umfasst:

   (a) eine Restorer DNA, die für ein Protein codiert, welches ein Inhibitor für eine Ribonuclease ist, und
   (b) einen ersten Promotor, der die Expression in mindestens spezifischen Zellen der Blüte, das Samens und/oder des Keimes einer Pflanze bestimmt, und worin die Restorer DNA in der gleichen transkriptionellen Einheit ist und unter der Kontrolle steht von diesem ersten Promotor.

2. Rekombinante DNA gemäß Anspruch 1, wobei der Inhibitor in der Lage ist, die Aktivität der extrazellulären Ribonuclease Barnase von **Bacillus amyloliguefaciens** zu neutralisieren.

3. Rekombinante DNA gemäß Anspruch 2, wobei der Inhibitor Barstar ist, mit der Aminosäuresequenz codiert gemäß der Sequenz in Figur 2, beginnend von der Nucleotid-Position 11.

4. Rekombinante DNA gemäß Anspruch 3, in der die Restorer DNA die codierende Sequenz, beginnend von der Nucleotid-Position 11 in Figur 2 umfasst.

5. Rekombinante DNA gemäß Anspruch 4, bei der die Restorer DNA das ClaI-HindIII Fragment der Figur 2 ist.

6. Rekombinante DNA gemäß einem der Ansprüche 1 bis 5, das weiterhin eine sekundäre chimäre DNA umfasst, umfassend:

   (c) eine Marker-DNA, die für eine Marker-RNA, ein Marker-Protein oder Marker-Polypeptid codiert, die/das, wenn sie in mindestens einem spezifischen Gewebe, oder mindestens in spezifischen Zellen der Pflanze vorhanden ist/sind, es erlaubt, diese Pflanze einfach von anderen Pflanzen zu trennen, die diese Marker-RNA, -Protein oder -Polypeptid in dem spezifischen Geweben oder den spezifischen Zellen nicht enthält; und
   (d) einen zweiten Promotor, der die Expression dieser Marker-DNA in mindestens einem spezifischen Gewebe oder in spezifischen Zellen bestimmt; diese Marker-DNA ist in der gleichen transkriptionellen Einheit und unter Kontrolle dieses zweiten Promotors.

7. Rekombinante DNA gemäß Anspruch 6, in der die Marker-DNA ein Protein oder Polypeptid codiert, die einem spezifischen Gewebe oder spezifischen Zellen eine Farbe verleiht; oder ein Protein oder Polypeptid codiert, die der Pflanze eine Toleranz gegenüber Stress, einer Erkrankung oder eine Schädlingsresistenz oder eine bakterielle Resistenz verleiht.

8. Rekombinante DNA gemäß Anspruch 7, wobei die Marker-DNA ein **Bacillus thuringiensis** Endotoxin codiert, das eine Insektenbständigkeit verleiht, oder ein bakterielles Peptid codiert, das eine bakterielle Resistenz verleiht.

9. Rekombinante DNA gemäß Anspruch 6, wobei die Marker-DNA ein modifiziertes Targetenzym für ein Herbicid mit einer niedrigeren Affinität für das Herbicid als das nicht modifizierte Targetenzym codiert.

10. Rekombinante DNA gemäß Anspruch 9, wobei die Marker-DNA ein Protein oder Polypeptid codiert, das ausgewählt ist aus der Gruppe einer modifizierten 5-Enolpyruvylshikimate-3-phosphatsynthase als Ziel des Herbicids, Glyphosat und einer modifizierten Glutaminsynthetase als ein Ziel für einen Glutaminsynthetase-Inhibitor, einschließlich Phosphinothricin.

11. Rekombinante DNA gemäß Anspruch 6, wobei die Marker-DNA ein Protein oder Polypeptid inhibiert, das die Aktivität eines Herbicids inhibiert oder neutralisiert.

12. Rekombinante DNA gemäß Anspruch 11, wobei die Marker-DNA ein Protein oder Polypeptid codiert, das eine Resistenz gegenüber einem Glutaminsynthetase-Inhibitor einschließlich Phosphinothricin verleiht.

13. Rekombinante DNA gemäß Anspruch 12, wobei die Marker-DNA ein sfr- oder sfrv-Gen ist.

14. Rekombinante DNA gemäß einem der Ansprüche 6 bis 13, wobei der zweite Promotor ist: ein konstitutiver Promotor, ein durch Verletzung induzierter Promotor, ein Promotor, der die selektive Expression in Pflanzenzellen, die eine photosynthetische Aktivität aufweisen, bestimmt, oder ein Promotor, der selektiv die Genexpression in

Blattzellen, Blütenzellen oder Samenzellen bestimmt.

15. Rekombinante DNA gemäß Anspruch 14, wobei der zweite Promotor ein 35S-Promotor, ein 35S'3-Promotor, ein Pnos-Promotor, ein TR1'- oder TR2'-Promotor oder ein SSU-Promotor ist.

16. Rekombinante DNA gemäß einem der Ansprüche 1 bis 15, der weiterhin umfasst:

(e) eine erste DNA, die ein Signalpeptid codiert, das in der Lage ist, diesen Inhibitor in Chloroplast oder Mitochondrien der Staubblattzellen zu transportieren; diese erste DNA ist in der gleichen transkriptionellen Einheit wie die Restorer DNA und der erste Promotor und zwischen dieser Restorer DNA und dem ersten Promotor; und/oder
(f) eine zweite DNA, die ein Signalpeptid codiert, das in der Lage ist, dieses Markerprotein oder Polypeptid in einen Chloroplast oder Mitochondrium von mindestens einem spezifischen Gewebe oder spezifischen Zellen zu transportieren; diese zweite DNA ist in der gleichen transkriptionellen Einheit wie die Marker-DNA und der zweiten Promotor und zwischen der Marker-DNA und dem zweiten Promotor.

17. Rekombinante DNA gemäß einem der Ansprüche 1 bis 16, in der der erste Promotor ein konstitutiver Promotor ist.

18. Rekombinante DNA gemäß einem der Ansprüche 1 bis 16, in der der erste Promotor die Expression mindestens in den Zellen der männlichen Organe der Pflanzen bestimmt.

19. Rekombinante DNA gemäß gemäß Anspruch 18, in der der erste Promotor selektiv die Expression in Staubblattzellen der Pflanzen bestimmt.

20. Rekombinante DNA gemäß einem der Ansprüche 18 oder 19, in der der erste Promotor die Expression in eine oder mehreren Staubblatt-Zellarten bestimmt, ausgewählt aus der Gruppe von Staubbeutel-, Pollen- und Filamentzellen.

21. Rekombinante DNA gemäß Anspruch 20, in der der erste Promotor die Expression in Staubbeutelzellen bestimmt.

22. Rekombinante DNA gemäß Anspruch 21, in der der erste Promotor ein Promotor von einem endogenen Pflanzengen ist, ausgewählt aus der Gruppe des TA29-Gens von Tabak, TA26-Gens von Tabak, TA13-Gens von Tabak, eines Gens, codierend für eine mRNA, die mit dem TA29-Gen hybridisierbar ist, eines Gen, codierend für eine mRNA, die mit dem TA13-Gen hybridisierbar ist, und eines Gens, codierend für eine mRNA, die mit dem TA26-Gen hybridisierbar ist.

23. Rekombinante DNA gemäß Anspruch 22, in der der erste Promotor der TA29-Promotor ist, der in dem NcoI-HindIII-Fragment des Plasmids pMB3, DSM 4470 enthalten ist.

24. Rekombinante DNA gemäß einem der Ansprüche 1 bis 16, in der der erste Promotor die Expression in mindestens den Zellen der weiblichen Organe einer Pflanze bestimmt.

25. Rekombinante DNA gemäß Anspruch 24, in der der erste Promotor selektiv die Expression in den Zellen der weiblichen Organe einer Pflanze bestimmt.

26. Rekombinante DNA gemäß Anspruch 24 oder 25, in der der erste Promotor die Expression in einer oder mehreren Zellarten der weiblichen Organe bestimmt, ausgewählt aus der Gruppe der Fruchtknoten-, Ovarum-, Stylus-, Narben- oder Septum-Zellen.

27. Rekombinante DNA gemäß Anspruch 26, in der der erste Promotor ein Promotor ist, der die Expression in den Stylus- und/oder Narbenzellen bestimmt, wie der Promotor des endogenen STGM4B12-Gens des Tabaks, des endogenen STGM3C9-Gens des Tabaks, der Promotor des endogenen STGM07-Gens des Tabaks oder der Promotor des endogenen STGM08-Gens des Tabaks.

28. Rekombinante DNA gemäß einem der Ansprüche 1 bis 27, die ein Teil des nuklearen Genoms der Zellen einer Pflanze oder eines Samens ist.

29. Rekombinante DNA gemäß Anspruch 28, die weiterhin eine DNA codierend für die Ribonuclease enthält.

30. Zelle oder Pflanze, die die rekombinante DNA gemäß einem der Ansprüche 1 bis 29 enthält.

31. Zelle gemäß Anspruch 30, die zu einer Pflanze ausgebildet werden kann.

32. Pflanze, die die DNA gemäß einem der Ansprüche 1 bis 29 enthält.

33. Pflanze, die die rekombinante DNA gemäß einem der Ansprüche 1 bis 29 in ihren gesamten Zellen enthält.

34. Pflanzensamen, der die rekombinante DNA gemäß einem der Ansprüche 1 bis 29 enthält.

35. Samen gemäß Anspruch 34, der ein Hybridsamen ist.

36. Pflanze, die integriert in ihre nukleare DNA ihrer Zellen enthält, die rekombinante DNA gemäß einem der Ansprüche 17 bis 23, und die in der Lage ist, männliche fertile Nachfolgepflanzen herzustellen, die die Ribonuclease und den Inhibitor dieser Ribonuclease in den spezifischen Zellen herstellt, wenn sie mit einer zweiten Pflanze gekreuzt wird, die eine zweite rekombinante DNA enthält, umfassend eine Sterilitäts-DNA, codierend für die Ribonuclease unter der Kontrolle eines Sterilitätspromotors, der selektiv die Expression in spezifischen Zellen der Staubblattzellen dieser zweiten Pflanze bestimmt, und die männlich steril ist aufgrund der selektiven Herstellung dieser Ribonuclease in diesen spezifischen Staubblattzellen.

37. Pflanze gemäß Anspruch 36, die homozygot ist für diese rekombinante DNA.

38. Pflanze, die integriert in ihre nukleare DNA aller Zellen enthält:

a) die rekombinante DNA gemäß einem der Ansprüche 17 bis 23, und
b) eine zweite rekombinante DNA, umfassend eine Sterilitäts-DNA, die diese Ribonuclease unter der Kontrolle eines Sterilitäts-Promotors, der die selektive Expression in spezifischen Staubblattzellen dieser Pflanze bestimmt, codiert

und die männlich fertil ist aufgrund der Neutralisation der Aktivität dieser Ribonuclease in diesen spezifischen Staubblattzellen durch den Inhibitor, hergestellt durch Expression der Restorer DNA in mindestens diesen spezifischen Staubblattzellen.

39. Pflanze gemäß Anspruch 38, die eine Hybridpflanze ist.

40. Pflanze gemäß einem der Ansprüche 36 bis 39, in der die Ribonuclease eine Barnase ist, und die Restorer DNA Barstar codiert.

41. Pflanze gemäß einem der Ansprüche 36 bis 40, in der der Sterilitätspromotor die Expression in eine oder mehrere Staubblattzellarten, ausgewählt aus der Gruppe der Staubbeutel-, Pollen- oder Filamentzellen, bestimmt.

42. Pflanze gemäß Anspruch 41, in der der Sterilitätspromotor die Expression von einer oder mehreren Arten der Staubblattzellen, ausgewählt aus der Gruppe der Tapetum- oder Staubbeutel-epidermalen Zellen bestimmt.

43. Pflanze gemäß Anspruch 42, in der der Sterilitätspromotor ein Promotor eines endogenen Pflanzengens, ausgewählt aus der Gruppe des TA29-Gens von Tabak, des TA26-Gens von Tabak, des TA13-Gens von Tabak, eines Gens, codierend für eine mRNA, hybridisierbar mit dem TA29-Gen, eines Gens, codierend für eine mRNA, hybridisierbar mit dem TA13-Gen und eines Gens, codierend für eine mRNA, hybridisierbar mit dem TA26-Gen, ist.

44. Pflanze gemäß Anspruch 43, in der der Sterilitätspromotor der TA29-Promotor, enthaltend in dem NcoI-HindIII-Fragment des Plasmids pMB3, DSM 4470 ist.

45. Pflanze gemäß einem der Ansprüche 36 bis 44, in der der erste Promotor und der Sterilitätspromotor der gleiche sind.

46. Mutter-Pflanzenpaar zur Herstellung von Samen, umfassend:

(a) eine männlich-sterile Mutterpflanze, die in ihr nukleares Genom aller Zellen incorporiert enthält: eine Ste-

rilitäts-DNA, codierend für die Ribonuclease unter der Kontrolle eines Sterilitätspromotors, der die Expression dieser Sterilitäts-DNA selektiv in spezifischen Staubzellen dieser Pflanze bestimmt und
(b) eine männlich-fertile Mutterpflanze, die incorporiert in ihre nukleare DNA aller Zellen enthält: die rekombinante DNA gemäß einem der Ansprüche 17 bis 23, wobei der erste Promotor die Expression in mindestens den gleichen spezifischen Staubblattzellen, die der Sterilitätspromotor bestimmt; und

wobei die männlich-sterile und männlich-fertile Pflanze gekreuzt werden können, um männlich-fertile Nachfolger zu produzieren, und umfassend die rekombinante DNA und die Sterilitäts-DNA unter der Kontrolle des Sterilitätspromotors.

47. Paar gemäß Anspruch 46, in dem die Ribonuclease Barnase ist und die Restorer DNA Barstar codiert.

48. Paar gemäß Anspruch 46 oder 47, in dem der Sterilitätspromotor die Expression in eine oder mehreren Staubblattzellarten, ausgewählt aus der Gruppe der Staubbeutel-, Pollen und Filamentzellen, bestimmt.

49. Paar gemäß Anspruch 48, in dem der Sterilitätspromotor die Expression in eine oder mehreren Staubblattzellearten bestimmt, ausgewählt aus der Gruppe Tapetum- und Staubbeutel-Epidermalzellen.

50. Paar gemäß Anspruch 49, in dem der Sterilitätspromotor ein Promotor eines endogenen Pflanzengens, ausgewählt aus der Gruppe des TA29-Gens von Tabak, des TA26-Gens von Tabak, des TA13-Gens von Tabak, eines Gens, codierend für eine mRNA, hybridisierbar mit dem TA29-Gen, eines Gens, codierend für eine mRNA, hybridisierbar mit dem TA13-Gen und eines Gens, codierend für eine mRNA, hybridisierbar mit dem TA26-Gen, ist.

51. Paar gemäß Anspruch 50, in dem der Sterilitätspromotor der TA29-Promotor, enthaltend in dem NcoI-HindIII-Fragment des Plasmids pMB3, DSM 4470 ist.

52. Paar gemäß einem der Ansprüche 46 bis 51, in dem der erste Promotor und der Sterilitätspromotor der gleiche sind.

53. Paar gemäß einem der Ansprüche 46 bis 52, in dem die männlich-fertile Mutterpflanze homozygot für die rekombinante DNA ist.

54. Paar gemäß einem der Ansprüche 46 bis 53 zur Herstellung von Hybridsamen.

55. Verfahren zur Herstellung einer transgenen Pflanze oder von Reproduktionsmaterial oder von Nachfolgerpflanzen davon, das die folgenden Schritte umfasst:

a) Transformieren einer Ausgangszelle einer Pflanze mit der rekombinanten DNA gemäß einem der Ansprüche 1 bis 27, um eine transformierte Pflanzenzelle herzustellen, die die rekombinante DNA stabil integriert in die nukleare DNA enthält;
b) Regenerieren dieser transgenen Pflanze aus dieser transformierten Zelle, und gegebenfalls
c) Propagieren dieser transgenen Pflanze, um das Reproduktionsmaterial oder Nachfolgerpflanzen zu erhalten, die die rekombinante DNA enthalten.

56. Pflanze, die integriert in ihre nukleare DNA ihrer Zellen enthält die rekombinante DNA gemäß Anspruch 17 oder einem der Ansprüche 24 bis 27, und die in der Lage ist, weibliche fertile Nachfolgepflanzen herzustellen, die die Ribonuclease und den Inhibitor dieser Ribonuclease in den spezifischen Zellen herstellt, wenn sie mit einer zweiten Pflanze gekreuzt wird, die eine zweite rekombinante DNA enthält, umfassend eine Sterilitäts-DNA, codierend für die Ribonuclease unter der Kontrolle eines Sterilitätspromotors, der selektiv die Expression in spezifischen Zellen des weiblichen Organs dieser zweiten Pflanze bestimmt, und die weiblich steril ist aufgrund der selektiven Herstellung dieser Ribonuclease in diesen spezifischen Zellen.

57. Pflanze gemäß Anspruch 56, die die rekombinante DNA homozygot enthält.

58. Pflanze, die integriert in ihre nukleare DNA ihrer gesamten Zellen enthält:

a) die rekombinante DNA gemäß Anspruch 17 oder einem der Ansprüche 24 bis 27, und
b) eine zweite rekombinante DNA, umfassend eine Sterilitäts-DNA, codierend für die Ribonuclease unter der Kontrolle eines Sterilitäts-Promotors, der die selektive Expression in spezifischen Zellen der weiblichen Or-

gane dieser Pflanze bestimmt, und die weiblich-fertil ist aufgrund der Neutralisierung der Aktivität dieser Ribonuclease in diesen spezifischen Zellen durch den Inhibitor, hergestellt durch die Expression der Restorer-DNA in mindestens diesen spezifischen Zellen.

59. Pflanze gemäß Anspruch 58, die eine Hybridpflanze ist.

60. Pflanze gemäß einem der Ansprüche 56 bis 59, in der die Ribonuclease Barnase ist und die Restorer DNA für Barstar codiert.

61. Pflanze gemäß einem der Ansprüche 56 bis 60, in der der Sterilitätspromotor die Expression in eine oder mehreren Zellarten, ausgewählt aus der Gruppe der Fruchtknoten-, Ovarium-, Stylus-, Narben- oder Septumzellen bestimmt.

62. Pflanze gemäß Anspruch 61, in der der erste Promotor ein Promotor ist, der die Expression in den Stylus- und/oder Narbenzellen bestimmt, wie der Promotor des endogenen STGM4B12-Gens von Tabak, des endogenen STGM3C9-Gens von Tabak, der Promotor des endogenen STGM07-Gens von Tabak oder der Promotor des endogenen STGM08-Gens von Tabak.

63. Pflanze gemäß einem der Ansprüche 56 bis 62, in der der erste Promotor und der Sterilitätspromotor der gleiche sind.

64. Mutter-Pflanzenpaar zur Herstellung von Samen, umfassend:

(a) eine weiblich-sterile Mutterpflanze, die in ihr nukleares Genom aller Zellen incorporiert enthält:
eine Sterilitäts-DNA, codierend für die Ribonuclease unter der Kontrolle eines Sterilitätspromotors, der die Expression dieser Sterilitäts-DNA selektiv in spezifischen Zellen der weiblichen Organe dieser Pflanze bestimmt und
(b) eine weiblich-fertile Mutterpflanze, die incorporiert in ihre nukleare DNA aller Zellen enthält: die rekombinante DNA gemäß Anspruche 17 oder einem der Ansprüche 24 bis 27, wobei der erste Promotor die Expression in mindestens den gleichen spezifischen Zellen der weiblichen Organe, die der Sterilitätspromotor bestimmt; und

wobei die weiblich-sterile und weiblich-fertile Pflanze gekreuzt werden können, um weiblich-fertile Nachfolger zu produzieren, und umfassend die rekombinante DNA und die Sterilitäts-DNA unter der Kontrolle des Sterilitätspromotors.

65. Paar gemäß Anspruch 64, in dem die Ribonuclease Barnase ist und die Restorer DNA Barstar codiert.

66. Paar gemäß Anspruch 64 oder 65, in dem der Sterilitätspromotor die Expression in eine oder mehreren Zellarten, ausgewählt aus der Gruppe der Fruchtknoten-, Ovarum-, Styrus-, Narben- und Septumzellen bestimmt.

67. Paar gemäß Anspruch 66, in dem der Sterilitätspromotor ein Promotor eines endogenen Pflanzengens ist, der die Expression in den Stylus- und/oder Narbenzellen bestimmt, wie der Promotor des endogenen STGM4B12-Gens von Tabak, des endogenen STGM3C9-Gens von Tabak, der Promotor des endogenen STGM07-Gens von Tabak oder der Promotor des endogenen STGM08-Gens von Tabak.

68. Paar gemäß einem der Ansprüche 64 bis 67, in dem der erste Promotor und der Sterilitätspromotor die gleichen sind.

69. Paar gemäß einem der Ansprüche 64 bis 68, in dem die weiblich-fertile Mutterpflanze homozygot für die rekombinante DNA ist.

70. Paar gemäß einem der Ansprüche 64 bis 69 zur Herstellung von Hybridsamen.

71. Verwendung der rekombinanten DNA gemäß einem der Ansprüche 1 bis 28 zur Herstellung eines Inhibitors einer Ribonuclease in mindestens spezifischen Zellen der Blüte, des Samens und/oder des Keimlings einer Pflanze.

72. Verwendung gemäß Anspruch 71, um die Aktivität dieser Ribonuclease, hergestellt in mindestens diesen spezifischen Zellen, zu neutralisieren.

Revendications

1. ADN recombinant comprenant un premier ADN chimérique, qui comprend:

   a) un ADN restaurateur qui code une protéine qui est un inhibiteur d'une ribonucléase, et
   b) un premier promoteur qui dirige l'expression au moins dans des cellules spécifiques d'une fleur, d'une semence et/ou d'un embryon de plante,

   et dans lequel ledit ADN restaurateur se trouve dans la même entité transcriptionnelle que ledit premier promoteur et sous le contrôle de ce dernier.

2. ADN recombinant selon la revendication 1, dans lequel ledit inhibiteur est capable de neutraliser l'activité de la ribonucléase barnase extracellulaire de Bacillus amyloliquefaciens.

3. ADN recombinant selon la revendication 2, dans lequel ledit inhibiteur est le barstar avec une séquence d'acides aminés codée par la séquence de codage qui commence à la position du nucléotide de la figure 2.

4. ADN recombinant selon la revendication 3, dans lequel ledit ADN restaurateur comprend la séquence de codage qui commence à la position du nucléotide 11 de la figure 2.

5. ADN recombinant selon la revendication 4, dans lequel ledit ADN restaurateur est le fragment ClaI-HindIII de la figure 2.

6. ADN recombinant selon l'une quelconque des revendications 1 ou 5, qui comprend également un deuxième ADN chimérique qui comprend:

   c) un ADN marqueur qui code un ARN marqueur, une protéine ou un polypeptide qui, lorsqu'ils sont présents au moins dans un tissu spécifique ou au moins dans des cellules spécifiques d'une plante, rendent ladite plante aisément séparable d'autres plantes qui ne contiennent pas ledit ARN marqueur, ladite protéine ou ledit polypeptide marqueurs dans ledit tissu spécifique ou dans lesdites cellules spécifiques; et
   d) un deuxième promoteur capable de diriger l'expression dudit ADN marqueur au moins dans ledit tissu spécifique ou dans lesdites cellules spécifiques;

   ledit ADN marqueur étant situé dans la même unité transcriptionnelle que ledit deuxième promoteur et sous le contrôle de ce dernier.

7. ADN recombinant selon la revendication 6, dans lequel ledit ADN marqueur code une protéine ou un polypeptide qui confèrent une couleur au moins audit tissu spécifique ou auxdites cellules spécifiques; ou qui code une protéine ou un polypeptide qui confèrent à ladite plante une tolérance vis-à-vis du stress, une résistance à une maladie ou à un insecte nuisible, ou une résistance bactérienne.

8. ADN recombinant selon la revendication 7, dans lequel ledit ADN marqueur code une endotoxine de *Bacillus thuringiensis* qui confère une résistance aux insectes, ou code un peptide bactéricide qui confère une résistance bactérienne.

9. ADN recombinant selon la revendication 6, dans lequel ledit ADN marqueur code une enzyme cible modifiée pour un herbicide, qui présente une plus faible affinité pour l'herbicide que l'enzyme cible non modifiée.

10. ADN recombinant selon la revendication 9, dans lequel ledit ADN marqueur code une protéine ou un polypeptide qui est sélectionné dans le groupe d'une 5-énolpyruvylshikimate-3 phosphate synthase modifiée qui sert de cible au glyphosate herbicide et une glutamine synthétase modifiée qui sert de cible à un inhibiteur de glutamine synthétase, incluant la phosphonothricine.

11. ADN recombinant selon la revendication 6, dans lequel ledit ADN marqueur code une protéine ou un polypeptide qui inhibent ou neutralisent l'activité d'un herbicide.

12. ADN recombinant selon la revendication 11, dans lequel ledit ADN marqueur code une protéine ou un polypeptide qui confèrent une résistance à un inhibiteur de la glutamine synthétase, incluant la phosphinothricine.

13. ADN recombinant selon la revendication 12, dans lequel ledit ADN marqueur est un gène sfr ou sfrv.

14. ADN recombinant selon l'une quelconque des revendications 6 ou 13, dans lequel ledit deuxième promoteur est un promoteur constitutif, un promoteur inductible à être induit par une lésion, un promoteur qui dirige l'expression de manière sélective dans des tissus de plante qui présentent une activité de photosynthèse ou un promoteur qui dirige l'expression d'un gène de manière sélective dans des cellules de feuille, des cellules de pétale ou des cellules de semence.

15. ADN recombinant selon la revendication 14, dans lequel ledit deuxième promoteur est un promoteur 35S, un promoteur 35S'3, un promoteur Pnos, un promoteur TR1' ou TR2' ou un promoteur SSU.

16. ADN recombinant selon l'une quelconque des revendications 1 ou 15, qui comprend également :

(e) un premier ADN qui code un peptide de transfert capable de transporter ledit inhibiteur dans un chloroplaste ou une mitochondrie desdites cellules d'étamine; ledit premier ADN étant situé dans la même unité transcriptionnelle que ledit ADN de restaurateur et que ledit premier promoteur, et entre ledit ADN de restaurateur et ledit premier promoteur; et/ou
(f) un deuxième ADN qui code un peptide de transfert capable de transporter ladite protéine ou ledit polypeptide marqueur dans un chloroplaste ou une mitochondrie d'au moins ledit tissu spécifique ou lesdites cellules spécifiques; ledit deuxième ADN étant situé dans la même unité transcriptionnelle que ledit ADN marqueur et que ledit deuxième promoteur, et entre ledit ADN marqueur et ledit deuxième promoteur.

17. ADN recombinant selon l'une quelconque des revendications 1 ou 16, dans lequel ledit premier promoteur est un promoteur constitutif.

18. ADN recombinant selon l'une quelconque des revendications 1 ou 16, dans lequel ledit premier promoteur dirige l'expression au moins dans des cellules de l'organe mâle d'une plante.

19. ADN recombinant selon la revendication 18, dans lequel ledit premier promoteur dirige l'expression de manière sélective dans les cellules d'étamine d'une plante.

20. ADN recombinant selon les revendications 18 ou 19, dans lequel ledit premier promoteur dirige l'expression dans un ou plusieurs types de cellules d'étamine sélectionnées dans le groupe des cellules d'anthère, de pollen et de filament.

21. ADN recombinant selon la revendication 20, dans lequel ledit premier promoteur dirige l'expression dans des cellules d'anthère.

22. ADN recombinant selon la revendication 21, dans lequel ledit premier promoteur est un promoteur provenant d'un gène endogène de plante sélectionné dans le groupe du gène TA29 du tabac, du gène TA26 du tabac, du gène TA13 du tabac, d'un gène qui code un ARNm apte à être hybridé avec ledit gène TA29, d'un gène qui code un ARNm apte à être hybridé avec ledit gène TA13 et un gène qui code un ARNm apte à être hybridé avec ledit gène TA26.

23. ADN recombinant selon la revendication 22, dans lequel ledit premier promoteur est le promoteur TA29 contenu dans le fragment NcoI-HindIII du plasmide pMB3, DSM 4470.

24. ADN recombinant selon l'une quelconque des revendications 1 ou 16, dans lequel ledit premier promoteur dirige l'expression au moins dans les cellules de l'organe femelle d'une plante.

25. ADN recombinant selon la revendication 24, dans lequel ledit premier promoteur dirige l'expression de manière sélective dans des cellules de l'organe femelle d'une plante.

26. ADN recombinant selon les revendications 24 ou 25, dans lequel ledit premier promoteur dirige l'expression dans un ou plusieurs types de cellules de l'organe femelle, sélectionnées dans le groupe des cellules d'ovaire, d'ovule, de style, de stigma ou de septum.

27. ADN recombinant selon la revendication 26, dans lequel ledit premier promoteur est un promoteur qui dirige l'ex-

pression dans les cellules de style et/ou de stigma, tel que le promoteur du gène endogène STGM4B12 du tabac, le promoteur du gène endogène STGM3C9 du tabac, le promoteur du gène endogène STGM07 du tabac ou le promoteur du gène endogène STGM08 du tabac.

28. ADN recombinant selon l'une quelconque des revendications 1 ou 27, qui fait part du génome nucléaire d'une cellule d'une plante ou d'une semence.

29. ADN recombinant selon la revendication 28, qui contient également un ADN qui code ladite ribonucléase.

30. Cellule d'une plante qui contient l'ADN recombinant selon l'une quelconque des revendications 1 à 29.

31. Cellule selon la revendication 30, qui peut être régénérée en une plante.

32. Plante qui contient l'ADN selon l'une quelconque des revendications 1 à 29.

33. Plante qui contient l'ADN recombinant selon l'une quelconque des revendications 1 à 29 dans toutes ses cellules.

34. Semence de plante dans laquelle ladite semence contient l'ADN recombinant selon l'une quelconque des revendications 1 à 29.

35. Semence selon la revendication 34, qui est une semence hybride.

36. Plante qui contient, intégré dans l'ADN nucléaire de toutes ses cellules, l'ADN recombinant selon l'une quelconque des revendications 17 à 23 et qui, est capable d'être croisée avec une deuxième plante qui contient un deuxième ADN recombinant comprenant un ADN de stérilité qui code ladite ribonucléase sous le contrôle d'un promoteur de stérilité qui dirige l'expression de manière sélective dans des cellules spécifiques d'étamine de ladite deuxième plante, et qui est un stérile mâle dû à la production sélective de ladite ribonucléase dans lesdites cellules spécifiques d'étamine, est capable de produire une progéniture de plantes mâles fertiles qui produisent ladite ribonucléase et ledit inhibiteur de ladite ribonucléase dans lesdites cellules spécifiques d'étamine.

37. Plante selon la revendication 36, qui est homozygote dudit ADN recombinant.

38. Plante qui contient, intégré dans l'ADN nucléaire de toutes ses cellules,

a) l'ADN recombinant selon l'une quelconque des revendications 17 à 23, et
b) un deuxième ADN recombinant comprenant un ADN de stérilité qui code ladite ribonucléase sous le contrôle d'un promoteur de stérilité qui dirige l'expression de manière sélective dans des cellules spécifiques d'étamine de ladite plante,

et qui est un fertile mâle grâce à la neutralisation de l'activité de ladite ribonucléase dans lesdites cellules spécifiques d'étamine par ledit inhibiteur produit par l'expression dudit ADN de restaurateur au moins dans lesdites cellules spécifiques d'étamine.

39. Plante selon la revendication 28, qui est une plante hybride.

40. Plante selon l'une quelconque des revendications 36 à 39, dans laquelle ladite ribonucléase est une barnase et ledit ADN de restaurateur code le barstar.

41. Plante selon l'une quelconque des revendications 36 à 40, dans laquelle ledit promoteur de stérilité dirige l'expression dans un ou plusieurs types de cellules d'étamine sélectionnées dans le groupe des cellules d'anthère, de pollen et de filament.

42. Plante selon la revendication 21, dans laquelle ledit promoteur de stérilité dirige l'expression dans un ou plusieurs types de cellules d'étamine sélectionnées dans le groupe des cellules d'épiderme de tapetum et d'épiderme.

43. Plante selon la revendication 42, dans laquelle ledit promoteur de stérilité est un promoteur provenant d'un gène endogène de plante sélectionné dans le groupe du gène TA29 du tabac, du gène TA26 du tabac, du gène TA13 du tabac, d'un gène qui code un ARNm apte à être hybridé avec ledit gène TA29, d'un gène qui code un ARNm

apte à être hybridé avec ledit gène TA13 et d'un gène qui code un ARNm apte à être hybridé avec ledit gène TA26.

44. Plante selon la revendication 43, dans laquelle ledit promoteur de stérilité est le promoteur TA29 contenu dans le fragment NcoI-HindIII du plasmide pMB3, DSM 4470.

45. Plante selon l'une quelconque des revendications 36 à 44, dans laquelle ledit premier promoteur et ledit promoteur de stérilité sont identiques.

46. Paire de plantes parentes destinées à produire des semences, comprenant:

(a) une plante parente mâle stérile qui contient, incorporé dans le génome nucléaire de toutes ses cellules, un ADN de stérilité qui code ladite ribonucléase sous le contrôle d'un promoteur de stérilité qui dirige l'expression dudit ADN de stérilité de manière sélective dans des cellules spécifiques de l'étamine de ladite plante; et
(b) une plante parente mâle fertile qui contient, incorporé dans l'ADN nucléaire de toutes ses cellules, l'ADN recombinant selon l'une quelconque des revendications 17 à 23, dans laquelle ledit premier promoteur dirige l'expression au moins dans les mêmes cellules spécifiques d'étamine que ledit promoteur de stérilité;

et dans laquelle ladite plante mâle stérile et ladite plante mâle fertile peuvent être croisées pour produire une progéniture mâle fertile comprenant ledit ADN recombinant et ledit ADN de stérilité, sous le contrôle dudit promoteur de stérilité.

47. Paire selon la revendication 46, dans laquelle ladite ribonucléase est la barnase et ledit ADN de réparation code le barstar.

48. Paire selon la revendication 46 ou 47, dans laquelle ledit promoteur de stérilité dirige l'expression dans un ou plusieurs types de cellules d'étamine sélectionnées dans le groupe des cellules d'anthère, de pollen et de filament.

49. Paire selon la revendication 48, dans laquelle ledit promoteur de stérilité dirige l'expression dans un ou plusieurs types de cellules d'étamine sélectionnées dans le groupe des cellules d'épiderme de tapetum et d'épiderme.

50. Paire selon la revendication 49, dans laquelle ledit promoteur de stérilité est un promoteur provenant d'un gène endogène de plante sélectionné dans le groupe du gène TA29 du tabac, du gène TA26 du tabac, du gène TA13 du tabac, d'un gène qui code un ARNm apte à être hybridé avec ledit gène TA29, d'un gène qui code un ARNm apte à être hybridé avec ledit gène TA13 et d'un gène qui code un ARNm apte à être hybridé avec ledit gène TA26.

51. Paire selon la revendication 50, dans laquelle ledit promoteur de stérilité est le promoteur TA29 contenu dans le fragment NcoI-HindIII du plasmide pMB3, DSM 4470.

52. Paire selon l'une quelconque des revendications 46 à 51, dans laquelle ledit premier promoteur et ledit promoteur de stérilité sont identiques.

53. Paire selon l'une quelconque des revendications 46 à 52, dans laquelle ladite plante parente mâle fertile est homozygote audit ADN recombinant.

54. Paire selon l'une quelconque des revendications 46 à 53, pour produire des semences hybrides.

55. Procédé de production d'une plante transgénique, d'un matériau de reproduction ou de plante provenant de ce matériau, qui comprend les étapes consistant à :

a) transformer une cellule de départ d'une plante avec l'ADN recombinant selon l'une quelconque des revendications 1 à 27, pour produire une cellule de plante transformée qui contient ledit ADN recombinant intégré de manière stable dans son ADN nucléaire;
b) régénérer ladite plante transgénique à partir de ladite cellule transformée, et facultativement,
c) propager ladite plante transgénique pour obtenir ledit matériau de reproduction ou ladite progéniture de plantes qui contiennent ledit ADN recombinant.

56. Plante qui contient, intégré dans l'ADN nucléaire de toutes ses cellules, l'ADN recombinant selon la revendication 17 ou l'une quelconque des revendications 24 à 27 et qui, est capable d'être croisée avec une deuxième plante

qui contient un deuxième ADN recombinant comprenant un ADN de stérilité qui code ladite ribonucléase sous le contrôle d'un promoteur de stérilité qui dirige l'expression de manière sélective dans des cellules spécifiques de l'organe femelle de ladite deuxième plante, et qui est un stérile femelle suite à la production sélective de ladite ribonucléase dans lesdites cellules spécifiques, est capable de produire une progéniture de plantes femelles fertiles qui produisent ladite ribonucléase et ledit inhibiteur de ladite ribonucléase dans lesdites cellules spécifiques.

57. Plante selon la revendication 56, qui est homozygote audit ADN recombinant.

58. Plante qui contient, intégré dans l'ADN nucléaire de toutes ses cellules,

a) l'ADN recombinant selon la revendication 17 ou l'une quelconque des revendications 24 à 27, et
b) un deuxième ADN recombinant comprenant un ADN de stérilité qui code ladite ribonucléase sous le contrôle d'un promoteur de stérilité qui dirige l'expression de manière sélective dans des cellules spécifiques de l'organe femelle de ladite plante,

et qui est un fertile femelle grâce à la neutralisation de l'activité de ladite ribonucléase dans lesdites cellules spécifiques par ledit inhibiteur produit par l'expression dudit ADN de restaurateur au moins dans lesdites cellules spécifiques.

59. Plante selon la revendication 58, qui est une plante hybride.

60. Plante selon l'une quelconque des revendications 56 à 59, dans laquelle ladite ribonucléase est la barnase et ledit ADN de réparation code le barstar.

61. Plante selon l'une quelconque des revendications 56 à 60, dans laquelle ledit promoteur de stérilité dirige l'expression dans un ou plusieurs types de cellules sélectionnées dans le groupe des cellules d'ovaire, d'ovule, de style, de stigma et de septum.

62. Plante selon la revendication 61, dans laquelle ledit premier promoteur est un promoteur qui dirige l'expression dans des cellules de style et/ ou de stigma, par exemple le promoteur du gène endogène STGM4B12 du tabac, le promoteur du gène endogène STGM3C9 du tabac, le promoteur du gène endogène STGM07 du tabac ou le promoteur du gène endogène SSTGM08 du tabac.

63. Plante selon l'une quelconque des revendications 56 à 62, dans laquelle ledit premier promoteur et ledit promoteur de stérilité sont identiques.

64. Paire de plantes parentes pour produire des semences, comprenant :

(a) une plante parente femelle stérile qui contient, incorporé dans le génome nucléaire de toutes ses cellules, un ADN de stérilité qui code ladite ribonucléase sous le contrôle d'un promoteur de stérilité qui dirige l'expression dudit ADN de stérilité de manière sélective dans des cellules spécifiques de l'organe femelle de ladite plante; et
(b) une plante parente femelle fertile qui contient, incorporé dans l'ADN nucléaire de toutes ses cellules, l'ADN recombinant selon la revendication 17 ou selon l'une quelconque des revendications 24 à 27, dans laquelle ledit'premier promoteur dirige l'expression au moins dans les mêmes cellules spécifiques de l'organe femelle que ledit promoteur de stérilité;

et dans laquelle ladite plante femelle stérile et ladite plante femelle fertile peuvent être croisées pour produire une progéniture femelle fertile comprenant ledit ADN recombinant et ledit ADN de stérilité, sous le contrôle dudit promoteur de stérilité.

65. Paire selon la revendication 64, dans laquelle ladite ribonucléase est la barnase et ledit ADN de réparation code le barstar.

66. Paire selon la revendication 64 ou 65, dans laquelle ledit promoteur de stérilité dirige l'expression dans un ou plusieurs types de cellules sélectionnées dans le groupe des cellules d'ovaire, d'ovule, de style, de stigma et de septum.

67. Paire selon la revendication 66, dans laquelle ledit promoteur de stérilité est un promoteur qui dirige l'expression dans les cellules de style et/ou de stigma, par exemple le promoteur du gène endogène STGM4B12 du tabac, le promoteur du gène endogène STGM3C9 du tabac, le promoteur du gène endogène STGM07 du tabac ou le promoteur du gène endogène STGM08 du tabac.

68. Paire selon l'une quelconque des revendications 64 à 67, dans laquelle ledit premier promoteur et ledit promoteur de stérilité sont identiques.

69. Paire selon l'une quelconque des revendications 64 à 68, dans laquelle ladite plante parente femelle fertile est homozygote audit ADN recombinant.

70. Paire selon l'une quelconque des revendications 64 à 69, pour produire des semences hybrides.

71. Utilisation de l'ADN recombinant selon l'une quelconque des revendications 1 à 28 pour produire un inhibiteur d'une ribonucléase au moins dans des cellules spécifiques d'une fleur, d'une semence et/ou d'un embryon d'une plante.

72. Utilisation selon la revendication 71 pour neutraliser l'activité de ladite ribonucléase produite dans au moins lesdites cellules spécifiques.

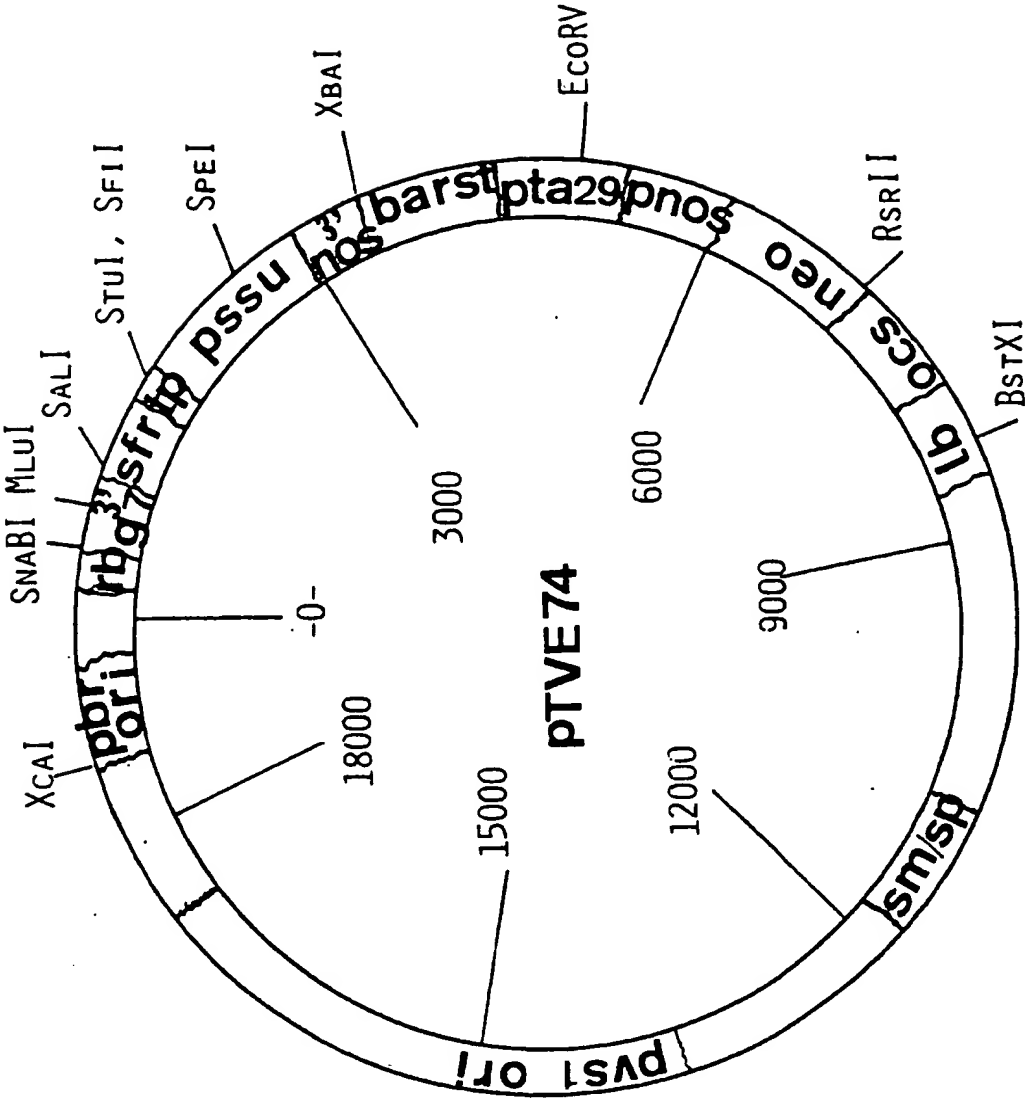
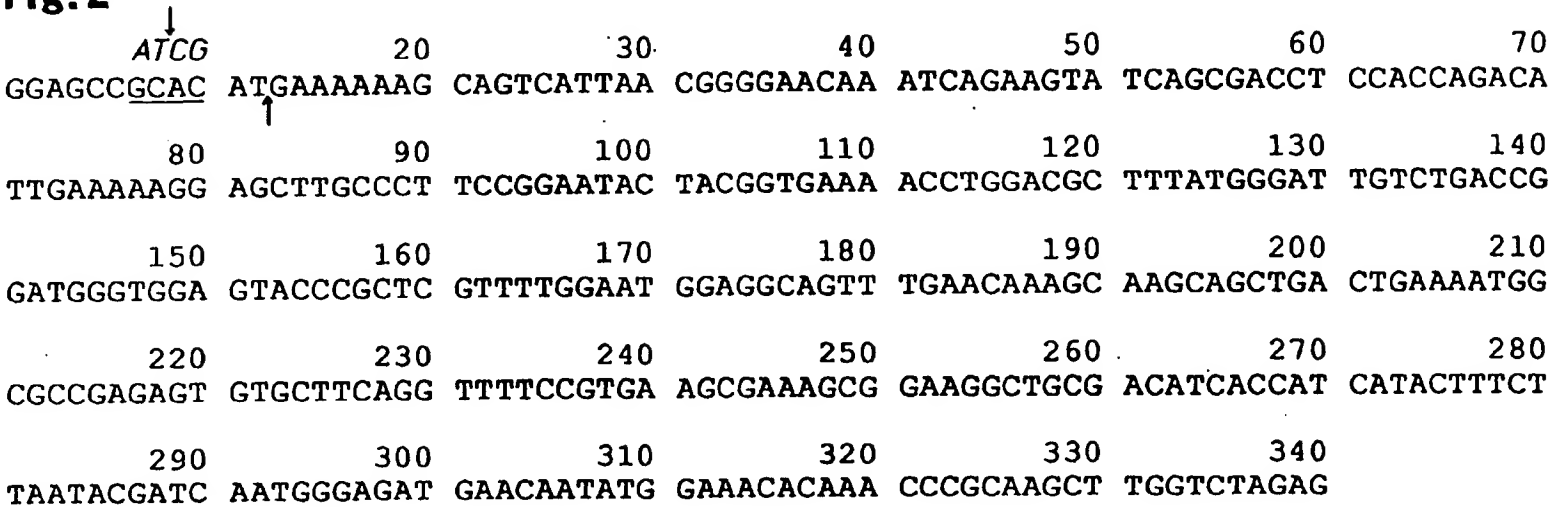

pTVE74
0
3000
6000
9000
12000
15000
18000
EcoRV
XbaI
SpeI
StuI, SfiI
SalI
SnaBI MluI
XcaI
RsrII
BstXI
pssu
3' nos
barst
pta29
pnos
neo
ocs
lb
ds/sp
pVS1 ori
pbr ori
rbg7
3'sfr

FIG. 1

Fig. 2



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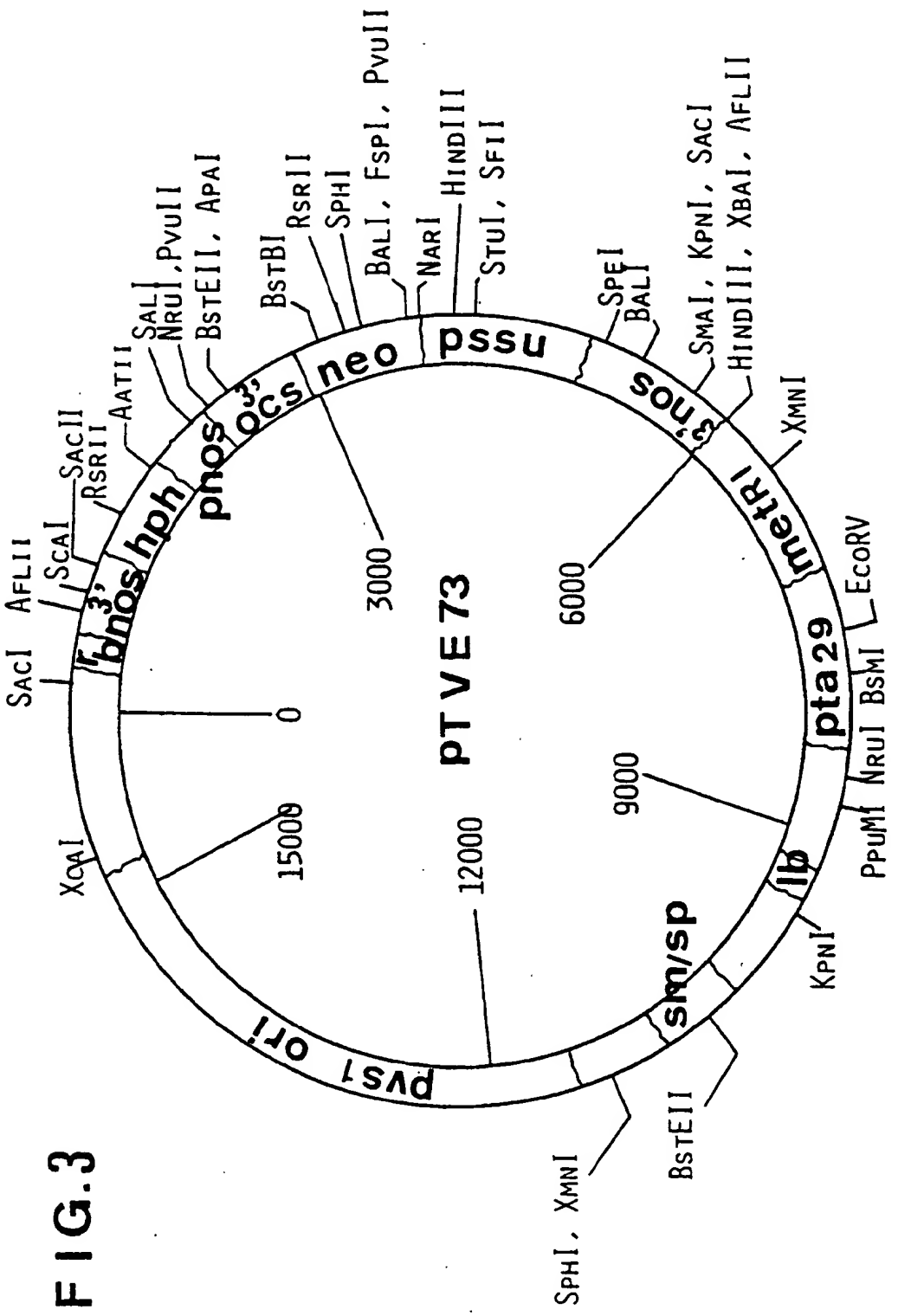
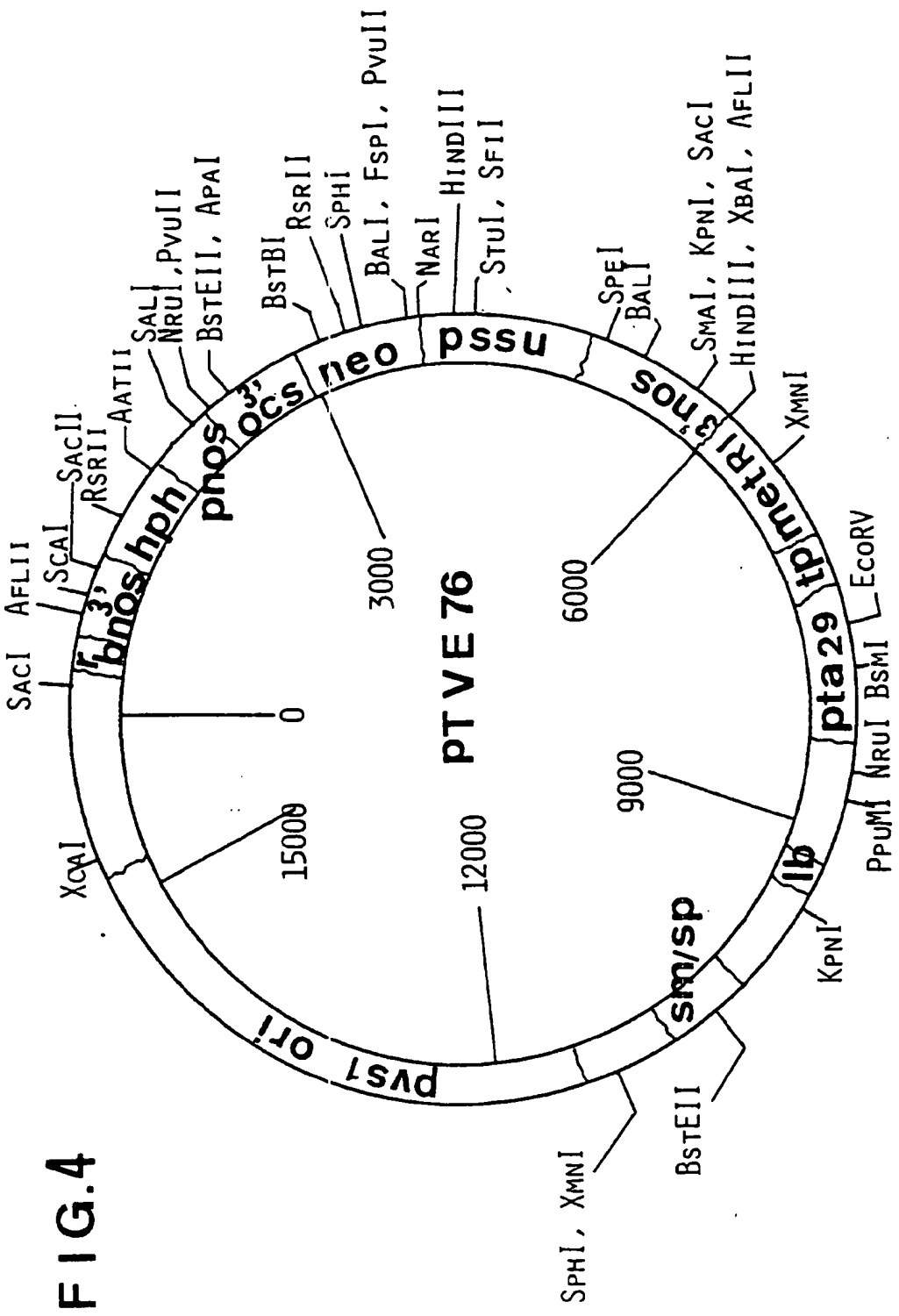

PTVE73
0
3000
6000
9000
12000
15000
pssu
neo
ocs 3'
phos
hph
3' bnos
pVS1 ori
sm/sp
lb
pta29
metR I
sou 2
SacI
AflII
ScaI
SacII
RsrII
AatII
SalI
NruI, PvuII
BstEII, ApaI
BstBI
RsrII
SphI
BalI, FspI, PvuII
NarI
HindIII
StuI, SfiI
SpeI
BalI
SmaI, KpnI, SacI
HindIII, XbaI, AflII
XmnI
EcoRV
BsmI
NruI
PpuMI
KpnI
BstEII
SphI, XmnI
XcaI

FIG.3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3000
6000
9000
12000
15000
pssu
neo
ocs 3'
phos
hph
3' nos
pVS1 ori
sm/sp
lb
pta29
tpl
metRI
3' nos
SacI
AflII
ScaI
SacII
RsrII
AatII
SalI
NruI, PvuII
BstEII, ApaI
BstBI
RsrII
SphI
BalI, FspI, PvuII
NarI
HindIII
StuI, SfiI
SpeI
BalI
SmaI, KpnI, SacI
HindIII, XbaI, AflII
XmnI
EcoRV
BsmI
NruI
PpuMI
KpnI
BstEII
SphI, XmnI
XcmI

FIG.4